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Toxicological Effect of Military Smokes and Obscurants on Aquatic Threatened and Endangered Species

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14. ABSTRACT

This research report describes actual field deposition and laboratory bench-scale applications of military smokes and obscurants (S&O) onto aquatic surfaces to study direct and indirect toxicological effects on threatened and endangered fish species, surrogate fish species, insect prey and aquatic plant habitat. Data allow predictions of impacts, effects, and mortality on relevant aquatic species from exposure to varying concentrations of S&O. Insect prey mortality is observed during relevant field deposition while heavier doses are required to induce lethal and sub-lethal effects on higher order organisms. Modes of toxicity include both direct contact of an organism with surface films and chemical transformation of S&O components into more toxic byproducts via photolytic mechanism upon exposure to sunlight. Recommendations for S&O field use are provided based on conservative estimates of chemical deposition from environmental release of S&O during military training exercises.

15. SUBJECT TERMS
military smokes and obscurants; aquatic threatened and endangered species, toxicity, *Daphnia magna*, *Ceriodaphnia dubia*, *Chironomus tentans*, Fountain darter (*Etheostoma fonticola)*, aquatic plants

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LIST OF ABBREVIATIONS AND ACRONYMS

Abbreviation	Definition
ACF	activated carbon filters
ACSIM	Assistant Chief of Staff for Installation Management
AEC	Army Environmental Command
ANOVA	analysis of variance
ANSI	American National Standards Institute
APG	Aberdeen Proving Ground
AR	Army Regulation
ASTM	American Society for Testing and Materials
CAA	Clean Air Act
CERL	Construction Engineering Research Laboratory
CI	confidence interval
DA	Department of the Army
DI	deionized (water)
DMSO	dimethyl sulfoxide
DoD	Department of Defense
DOI	Department of Interior
DW	dry weight
EAG	Edwards Aquifer Groundwater
ECBC	Edgewood Chemical and Biological Center
EL	Environmental Laboratory
EPCRA-TRI	Emergency Planning and Community Right to Know Act – Toxic Release Inventory
ERDC	Engineer Research and Development Center
ERDEC	Edgewood Development and Engineering Center
ESA	Endangered Species Act
FID	flame ionization detector
FLW	Fort Leonard Wood
FO	fog oil
FW	feedwater system
GC	gas chromatograph

Abbreviation	Definition
GC/MS	gas chromatograph/mass spectrometer
GFF	glass fiber filter
GIS	geographic information system
GSI	Gonado-somatic index
HOC	Home Oil Company
HPLC	high performance low chromatography
HQ	headquarters
HQUSACE	Headquarters, U.S. Army Corps of Engineers
IARC	International Agency for Research on Cancer
ID	inside diameter
INHS	Illinois Natural History Survey
ISSN	International Standards Serial Number
LC_{50}	lethal concentration (killing 50 percent of test subjects)
MACOM	Major Army Command
MHRS	moderately hard reconstituted synthetic (water)
MHRW	moderately hard reconstituted water
MS	mass spectrometer
NEPA	National Environmental Policy Act
NFHTC	National Fish Hatcheries Technical Center
NFO	new fog oil
NRC	National Research Council [of the National Academies of Science]
OFO	old fog oils
OMB	Office of Management and Budget
ORNL	Oak Ridge National Laboratory (fully spelled out in ref. citation)
PAH	polycyclic aromatic hydrocarbon
PEG	polyethelen glycol
PTFE	polytetrafluoroethylene
PVC	polyvinyl chloride
PVDF	hydrophilic polyvinylidene fluoride
PWTB	Public Works Technical Bulletin
S&O	smokes and obscurants

Abbreviation	Definition
SAR	used as a limitation on Form 298
SERDP	Strategic Environmental Research and Development Program
SGS	smoke generating system
SMH	synthetic moderately hard (water)
SMR	synthetic moderately hard water
TE	threatened, endangered
TEC	threatened, endangered, or federal candidate
TES	threatened and endangered species
TFE	tetrafluoroethylene
TOC	total organic carbon
TR	Technical Report
TRI	Toxic Release Inventory
UCH	unresolved complex humps
USACE	U.S. Army Corps of Engineers
USEPA	U.S. Environmental Protection Agency
UV	ultraviolet
UVB	ultraviolet narrow band
UVR	ultraviolet radiation
WAF	water accommodated fraction
WET	whole effluent toxicity
WSF	water soluble fractions
YCT	Yeast-Cereal Leaves – Trout

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EXECUTIVE SUMMARY

The U.S. Army must continually maintain a state of high readiness and alertness based on current geographical uncertainties. Preparation for adverse and unknown battlefield conditions requires military training activities using the cover of smokes and obscurants (S&O). S&O release active chemicals into the environment. The need to effectively quantify the emissions resulting from the use of S&O, and assess the potential health and environmental impact of these emissions, has become an important issue for the U.S. Army. The need for such data was first identified as a result of occurrences at the Massachusetts Military Reservation. Since that time other requirements, such as reporting under the Emergency Planning and Community Right to Know Act – Toxic Release Inventory (EPCRA-TRI), have also been identified. Additionally, for purposes of environmental stewardship, the impact of S&O on the vitality and survivability of threatened and endangered species (TES) must be ascertained. Threatened and endangered fish cohabit training areas where S&O are released. This report documents studies of S&O collection and chemical composition and the effects of the most important S&O — fog oil (FO) — on fish, cover plants, and insect prey relative to toxicity, fecundity, and food chain disruption.

Based on the data presented, each type of S&O examined has an optimal substrate material for collection. This work reaffirmed that military fog oils are very complex hydrocarbon mixtures composed of a multitude of chemically similar components. Our investigations showed that complete analysis and characterization is difficult, if not impossible. Two-dimensional chromatography, despite its enhanced resolution over one dimensional gas chromatography, is not able to completely characterize such samples.

While field deposition rates are variable, FO and other S&O deposition is generally undetectable beyond 50 m downwind of the source. While varying ambient field conditions pose a number of challenges to acute toxicity testing with FO, our results showed that acute toxicity to a common freshwater crustacean (*Daphnia magna*) was measurable under field conditions. Also while low levels of FO deposition did not have significant effects on a suite of variables related to midge development from larva to adult, a higher dose of oil resulted in decreased numbers of larvae pupating and successfully emerging from the water surface as adults.

Under field conditions we were unable to detect any acute toxicity at other trophic and phylogenetic levels involving green algae, submersed vascular plants, several species and genera of fish, and a common amphibian.

These studies showed that photolysis of FO on water can dramatically increase the toxicity of FO, and it increases the amount of water soluble components. At the FO levels observed in the field photolysis does not increase the toxic effects of the water beneath the oil layer, but at much higher FO concentrations the water become highly toxic. Fountain darter larvae are sensitive to relatively low concentrations (e.g., 10 ppm) of photolyzed FO in water. Darter adults, eggs, and juvenile fish are much less sensitive. The duration of this increased sensitivity is unknown, but nonetheless it is limited to the time of emergence from the egg until some point in physiological development to juvenile status.

Based upon the results of this study, we conclude that FO toxicity to aquatic organisms in the field, while measurable, is low and preventable provided that the generation point is located greater than 50 m from a water body containing TES or their prey items and aquatic plant cover. This distance protection can be enhanced by refraining from FO use during periods when larvae of endangered fish are most likely to be present. Our work showed that continued buildup of FO on a controlled volume of water can eventually result in a concentration that proves toxic to fish larvae, but this is unlikely in the field where continual water exchange occurs and the environmental water volume is much greater. Conservatively, we suggest that FO training exercises be limited to five consecutive days.

This work has resulted in the development of approaches that may be used by the military for the conservation and management of biological resources and systems, particularly those related to TES, when working with fog oil during field exercises. More specifically, this work resulted in the development of products and approaches that, a) may be applied to chemical and other stressor effects on TES; b) provides both TES and surrogate species specific data on military-specific chemical stressors; c) can assist U.S. Army and other biologists and natural resources managers in the preparation of biological assessments in accordance with the ESA; d) can provide endpoints and other data to assist military service biologists and natural resources managers in the preparation and implementation of required Endangered Species Management Plans; e) can assist the military services in the preparation of environmental assessments and environmental impact statements in accordance with the National Environmental Policy Act (NEPA) relative to the use, application, and effects of chemical stressors; f) can assist the military services biologists and natural resources managers with overall biological and natural resource management in accordance with the Sikes Act; g) can assist the military services in meeting water quality criteria for the protection of aquatic life in accordance with the Clean Water Act; h) can assist military service biologists, natural resources managers, and decision makers with risk assessment; and i) advances the scientific understanding of the how various airborne pollutants impact the survival, fecundity, food availability, and populations of sensitive threatened or endangered aquatic organisms. Overall the information provided in this report should influence S&O usage to ensure the continued survival of our nation's rare and endangered species while preventing interruption of military critical activities.

While the information contained in this report adds significantly to scientific understanding of the effects of military FO and colored smokes, significant knowledge gaps remain. Currently data on S&O effects are lacking or at best inadequate for major phylogenetic groups. For example, there are no data on S&O effects on reptiles and practically none for terrestrial plants and mammals. Additionally, while data presented in this report are adequate for the species used, other genera and families within the studied phyla (e.g., Ictalurids, emergent plants) may react entirely differently to S&O exposure. Thus, additional research involving additional species is needed. Additionally, data on the effects of the combined use of FO and other obscurant compounds such as graphite are meager for all phylogenetic groups. Research on potential additive or synergistic effects of combined S&O use is necessary. Also, further research on trophic transfer effects and mechanisms, and potential long-term effects is also necessary. Furthermore, research on potential effects of newly developed and deployed S&Os is necessary to ensure that these new training aids are not environmentally problematic.

1. OBJECTIVE

The overall objective of this work was to study direct and indirect effects of *actual field deposition* of the most common military smokes and obscurants (S&O) on relevant surrogate aquatic species for threatened and endangered insect-eating fish, threatened or endangered fish, the insect prey of these fish, and habitat plants using lethal and sub-lethal endpoints such as survivorship, growth, and fecundity. Data were obtained and analyzed to help predict the impacts, effects, and mortality on these relevant aquatic species from exposure to varying concentrations of S&O. The purpose of the work was to develop and refine approaches and information for use by the military services, government agencies, and the private sector to assist with and implement the conservation, management, and recovery of aquatic TES, aquatic ecosystems, and other resources.

This work supports U.S. Army and military obligations under various mandates including the Endangered Species Act, the Clean Water Act, and the National Environmental Policy Act. It directly addresses Statement of Need CSSON-03-01, "The Impact of Military Training Activities, Land Management Actions and Species/Habitat Sensitivities on Aquatic Threatened and Endangered Species." Fog oil was identified as the S&O material of highest usage and, therefore, of highest concern regarding the subject ecological niche. The first-year effort tested the toxicological effects of S&O deposition on aquatic organisms under realistic field conditions. The planned goal for subsequent project years was to test S&O release and toxicological effects under controlled laboratory conditions within an environmental chamber located at U.S. Army Engineer Research and Development Center – Construction Engineering Research Laboratory (ERDC-CERL).

Because U.S. military forces were actively engaged in combat during this project, S&O were largely unobtainable for release testing, as documented in quarterly project reports. This scarcity of necessary research materials had a major impact on project progress and accomplishments. This report describes the results from field testing as well as chemical testing and toxicological studies initiated with the small fog oil quantities present at the time in the laboratory and on the relevant organic dyes that are currently contained within military colored smoke grenades.

2. BACKGROUND

2.1 Problem Statement

In order to fulfill its role in national defense, the U.S. military services must continually maintain a state of high readiness and alertness based on current geopolitical uncertainties and other factors. Maintenance of appropriate states of readiness and alertness requires training, much of which must take place on U.S. military installations and under field conditions. Concurrent with a broad role in national defense, the U.S. military services also have obligations and a role in maintaining and preserving the nation's environmental security. Achieving a balance satisfying military mission and training needs, maintenance and conservation of training lands and the environmental resources thereon, and compliance with Congressional mandates and public expectations, requires sound management based on cogent scientific information.

The Endangered Species Act (ESA) prohibits any action that adversely affects TES and their habitats. When information and data on the effects of military actions and training on TES are lacking, performance is restricted so that liberal protection of TES is provided. The U.S. Army, as the Conservation Pillar lead for the U.S. military services, has identified investigation of the impacts of military training and operations on TES as an area of high importance (U.S. Army, 1999; 2000). The U.S. Army Threatened and Endangered Species Advisory Group, which is composed of HQ DA, MACOM, and installation program experts, has further identified military smokes and obscurants (S&O) as areas of priority focused research investigation.

Preparation and training for adverse and unknown battlefield conditions requires military training activities using S&O. Quantifying the emissions resulting from S&O use and assessing the potential health and environmental impact of these emissions has become a critical issue for the U.S. Army. The need for such data has been identified as a result of occurrences at the Massachusetts Military Reservation and other military installations. Since that time, other requirements, such as reporting under the Emergency Planning and Community Right to Know Act - Toxic Release Inventory (EPCRA-TRI) have also been identified. TES, notably fish and mussels, either cohabit or live in proximity to training areas where S&O are released; therefore, the impact of S&O on the vitality and survivability of aquatic TES must be ascertained. This work addresses U.S. Army user requirements for the effects of hazardous air pollutants and particulate matter on aquatic TES, and concurrent requirements of understanding of benthic communities, ecosystems, and organisms, (see SERDP CSSON-03-01) as well as U.S. Army requirements associated with emissions as defined under the Clean Air Act. This work will study the effects of S&O on fish, aquatic plants, and insect prey for toxicity, fecundity, and food chain disruption. These data will influence S&O usage to ensure the continued survival of our nation's rare and endangered biological resources while preventing interruption of military activities.

The five major S&O in common use are fog oil, graphite smoke, and yellow, green, and red signaling smokes. Use of any type of S&O necessitates an environmental release of the active chemical. This work will assess the effect on fish, insects and mussels for these disparate types of S&O when they are released into the environment. Deposition of these fogs and smokes onto the water will create a layer at the surface and could alter gas exchange at the air/water interface

while also directly impacting emerging insects. Dissolution into the water column will contaminate the media, potentially causing direct effects on fish, mussels and other benthic organisms, and aquatic stages of insects.

Our initial proposal focused on these five primary S&O. Fog oil (FO) is an obscurant used to create visually limiting conditions for field training and maneuvering. It is a light hydrocarbon containing hundreds of organic constituents (Getz et al., 1996; Katz et al., 1980). This oil will form a hydrophobic layer at the water surface and will impact organisms that intersect this layer. Graphite smoke is used as an infrared obscurant, dispersing fine particles of graphite into the air (NRC 1999a). Little is known regarding the environmental impact of graphite; however, it is not volatile and will be persistent. It will provide an example of particulate residue effects on aquatic TES. Colored smokes are used for screening troops from view, signaling, and marking field positions. Some formulations of these smokes have been known to contain, generate, and disperse toxic and carcinogenic chemicals into the environment (NRC 1999b). Colored smokes offer examples of specific organic chemical contamination in the aquatic environment.

After initial field experiments and due to insufficient S&O supplies, the research focus shifted to the primary obscurant used by the military — fog oil (FO). A commonly used visual obscurant, FO is a middle distillate petroleum oil that is vaporized at high temperature (Boiling Point = 300-600°C) by a mobile smoke generator mounted on a military vehicle (von Stackleberg et al., 2004). Fog oil is not manufactured specifically for military use, but is taken from stocks of industrial lubricant oil (Driver 1993). Upon exposure to the atmosphere, the vapors condense to form a white fog that is ejected over a wide field training area (NRC et al., 1997). This oil fog is carried downwind and the aerosol droplets deposit on environmental surfaces. This activity may present a hazard to threatened, endangered, or federal candidate (TEC) species which are known to exist on or near military installations where FO obscurant is used in practice maneuvers (Rubinoff 2005). While the effects of FO deposition onto and inhalation by red-winged blackbirds (*Agelaius phoeniceus)*, house sparrows (*Passer domesticus*), and red cockaded woodpeckers (*Picoides borealis*) have been studied (Driver et al. 2002a,b), few investigations have been conducted on the toxicity of FO to aquatic life (Poston et al., 1986, 1988).

While it is important to study the S&O in the atmosphere to understand inhalation effects on terrestrial species, aquatic species will be affected only by the fraction of the S&O cloud that settles and deposits on the water surface. For instance, FO will form a hydrophobic layer at the aquatic surface. The oil can then (1) affect oxygen transport into the water causing species stress, (2) contribute to the water soluble fraction of hydrocarbons in the water column, which has a direct toxic effect on fish, mussels, other benthic organisms, and the aquatic stages of insects, and (3) impact organisms that must pass the air/water interface during its lifecycle thereby necessitating also passing through the oil layer. It is critical, therefore, to assess the fraction of the S&O that can deposit on water surfaces from the atmospheric cloud, as this is the fraction that will have affect aquatic life.

In September 1997, the Director of the Army Staff directed the Assistant Chief of Staff for Installation Management (ACSIM) to establish a General Officer Steering committee to address the implications of the EPCRA-TRI, NEPA, the Clean Air Act (CAA), and health hazard assessments for the Army. The ACSIM has supported the development of a comprehensive program to

identify the emissions resulting from range operations including smoke and pyrotechnic devices, to assess the environmental and health hazard impacts resulting from their use.

The importance of U.S. military lands to TES and other species is well recognized (Boice, 1996; U.S. Army, 1995; U.S. Air Force, 1997; U.S. Navy, 1999; U.S. Marine Corps, 1998). Of approximately 220 TES on or affected by military lands (Boice, 1996), at least 170 occur on U.S. Army installations (U.S. Army, 2002). In addition, at least 80% of U.S. Army installations have TES on the installation proper and an even higher percentage of installations have an effect on TES habitats and ecosystems (U.S. Army, 2002). As an example, two mussels that have been identified as candidates for federal listing , the elktoe, *Alasmidonta marginata*, and the spectaclecase, *Cumberlandia monodonta*, as well as two identified candidate fish (the plains topminnow, *Fundulus sciadicus*, and the bluestripe darter, *Percina cymatotaenia*) occur within the boundaries of Fort Leonard Wood (FLW), Missouri (Sternburg et al., 1998). Therefore, there is reason for concern regarding the use of smokes and obscurants on major military training installations given that their aquatic impacts are largely unknown.

Nearly 300 freshwater mussels species of the family Unionidae are known to occur in North America; however, as many as 36 species have become extinct in recent times (Brown and Banks, 2001). This high rate of extinction can be related in part not only to benthic and other habitat changes brought about by military and other activities but also to pollution and water quality. In the southeastern United States alone, 13% of the known species had been extirpated by 1997, and 60% were threatened to some extent (Neves et al., 1998). While the picture is less bleak for fish, there is reason for concern for their diversity as well. For example, of the 217 species of fish known to occur in Virginia, 21% are in varying degrees of jeopardy at the state level (Burkhead and Jenkins, 1991).

Federal agencies are mandated to take no action that is likely to compromise species protected under the Endangered Species Act of 1973. However, land managers of military installations are required to provide a natural environment for military training (Sternburg et al., 1998). Because of the variety of potential TES that may inhabit the numerous military installations across the country, it is unreasonable to attempt to assess the effects of various obscurants on each individual TES. This work will study the effects of S&O on both surrogate and actual aquatic TES, under both field conditions and controlled laboratory experiments. We have used the fathead minnow (*Pimephales promelas*) as a surrogate for predatory fish, the fountain darter, *Etheostoma fonticola*; Topeka shiner, *Notropis topeka;* and rainbow trout, *Oncorhynchus mykiss*, all threatened or endangered fish, the freshwater cladoceran *Daphnia magna* and *Chironomus tentans* (a benthic dipteran) both food sources for TE fish, a representative amphibian (northern leopard frog, *Rana pipiens*) and the midge (*Chironomus tentans*), an insect food organism for TE fish. This report also summarizes the results of research on the exposure of aquatic plants that are used for shelter and food for TE fish, sago pondweed, *Stuckenia pectinatus*; and *Selenastrum capricornutum*, a green algae.

To perform assessments and understanding of the effect of S&O on aquatic TE organisms, we initially proposed the following tasks (in brief):

Task 1: Field Collection of S&O Chemical Deposition. Employment of S&O releases chemical vapors, aerosols, and particulates into the atmosphere. While a number of studies have focused on the chemical release to the atmosphere (U.S. Army, 2001), fewer examine the settleable fraction that may deposit on water surfaces. This task will measure deposition rates and analytical characterization of the deposits for the five S&O during *actual detonation and releas*e under outdoor field conditions at the Aberdeen Test Center. This task will (a) separate the settable emissions from actual S&O release from the volatile emissions that will not impact aquatic TES, (b) measure the relevant concentration ranges of residues that are released during a training scenario, and (c) measure the ability of water to act as a collection substrate.

Task 2. Field Exposure of Selected Organisms. We will subject selected organisms to deposition of residue from actual S&O released in the field. Populations of organisms in open containers will be positioned radially at various distances (from 5 to 800 meters) from a central S&O release point. S&O will be released for an amount of time equal to a training event or scenario. After exposure, the organisms will be observed for toxic effects. This task will measure adverse effects of S&O on the aquatic organisms under actual field release conditions

Task 3: Evaluation of Exposure Chamber. All laboratory fogging was done in a containment structure designed with several useful characteristics. A 10' wide x 10' deep x 7' high structure at ERDC-CERL functioned as a containment structure for smoke release. Chamber ventilation tied into existing ductwork for evacuation and emission control. Within the chamber, a smaller, secondary enclosure can hold smoke generation devices so that the amount of smoke released to the larger chamber can be controlled. This task will tightly control the S&O emissions and insure organisms have been dosed with environmentally relevant residue concentrations.

Task 4: Chamber Calibration and Control Experiments. Experiments were conducted to address and characterize several parameters before introduction of living species. First, the deposited smoke residue will be characterized for agreement with residue data obtained from the field in Task 1. Agreement will ensure the analytical procedures are adequate and that the known toxic and hazardous by-products are present in the residue. An online sampling technique will be tested to ensure accurate measurement of the smoke deposition pulse downstream from the exposure chamber. Differences in the flow rate and smoke deposition concentration will change the chemical exposure pulse width (exposure time) and height (exposure concentration) that the species will experience. Second, the smoke deposition rate from each S&O generation device must be well characterized while in the secondary enclosure to provide highly controlled deposition concentrations onto the water within the chamber. The final control experiment will observe the effects of photolysis on the chemical composition of the deposition. Based on data from Task 1 that studies the effect of sunlight on the chemical composition of the deposit, the chamber can be outfitted with solar simulators to photolyze the S&O residue.

Task 5: Direct and indirect effects of S&O on predatory fish and mussels.

2.2 Selection of Test Organisms

When considering the potential impacts of toxicants upon TES, freshwater mussels in particular, a variety of indirect effects must be taken into account in addition to potential direct effects. For

example, if larval midges, a common food insect for predatory fish, are highly susceptible to fog oil contamination, predatory fish populations will be indirectly affected through reduced prey availability. This impact in turn could affect mussel populations if the fish serves as a host for mussel glochidia (the larval mussel stage that is parasitic upon fish). Therefore, we propose to assess the impacts of environmentally relevant concentrations of S&O on the fathead minnow, *Pimephales promelas*, as a ubiquitous surrogate for predatory fish, on the greenback cutthroat trout, *Oncorhynchus clarkii stomias,* a threatened fish that inhabits streams on military installations, on juveniles of the rainbow mussel, *Villosa iris*, (endangered in some states, including Illinois and Wisconsin), and on midges, *Chironomus tentans,* as an example of an insect food organism for fish.

Subtask 1. Effects of S&O on midge larvae, pupae, adult emergence, and tissue residues. Late larval midges will be exposed in static and flow-through bioassays. Midge starter cultures will be obtained from a commercial source and then reared in the laboratory according to standardized methods (ASTM, 1995). Midge larvae in the static exposure treatments will be loaded into bioassay jars, which will be placed in the chamber for exposure to a particular S&O. This task will determine how various S&O affect the survival of the midge, *Chironomus tentans* and determine the effects of S&O on the midge's ability to perform the energetically demanding transformation from larva to pupa to adult, and to emerge from the water surface into the terrestrial environment.

Subtask 2. Effects of S&O on midge oviposition. In a separate experiment, aquaria containing water will be placed in the exposure chamber. After deposition of S&O on the water surface, aquaria will be carefully transferred to nearby environmental chambers along with unexposed aquaria as controls. Adult *C. tentans* (1-2 males and 5-10 females per aquarium, depending upon availability) will be placed into the aquaria. An ample airspace will be provided above the water surfaces within the aquaria to allow mating of the insects. Aquaria then will be monitored for 1) number of egg masses produced, and 2) number of larvae surviving after one week.

Subtask 3. Effects of S&O on fish. Less than 24-hour-old fathead minnows will be tested in both static and flowing (3-4 different velocities) exposures in a manner similar to that described above for midges. Toxicity endpoints for fish will include survival, weight gain, and egg hatching success. This task will determine how various S&O affect the survival of the TE fish and cyprinid surrogates for TE fish and determine how various S&O affect fish population dynamics (survival, egg viability, GSI).

Subtask 4. Effects of S&O on freshwater mussels. For the mussel exposures, we will use juvenile *V. iris* because they are more sensitive to toxicants than adults (Jacobson et al., 1993). Static and flow-through exposure of mussels will be similar to that described for midges except that smaller exposure vessels will be developed for placement into the exposure chamber and raceways because of the small size of the test organisms. In addition, mussels will be evaluated only for survival 48 hours after exposure.

3. MATERIALS AND METHODS

To accomplish the above listed tasks, the research naturally divided into several components that included field work and laboratory work. The following sections describe the materials and methods used to investigate the effects of S&O on aquatic TE organisms.

3.1 S&O Release and Deposition in the Field During Simulated Training Events

Field release experiments were conducted during May and August 2003 at Aberdeen Proving Ground, MD. A central release point was designated for each type of S&O. In all the experiments, the collection media were placed in a straight line at different distances downwind from the point of release. The FO was released using a generation system mounted on the back of a vehicle. The generator slowly dropped FO onto a heated surface, which vaporized the oil into small aerosol droplets. A blower then ejected the droplets from the generator, forming a dense white cloud. The graphite smoke was emitted in a similar manner, but since the graphite flakes were already in particulate form, no heat was needed. Figure 1 shows both the FO and graphite smoke being emitted simultaneously from the same vehicle. HOC FO (Home Oil Co., Cowarts, AL) was used in these experiments.

Figure 1. FO and graphite smoke generation from one vehicle; FO smoke is white and graphite flake smoke is black.

Colored smoke grenades emit bright clouds from a small handheld canister. The grenade tab was pulled and the grenade was set on the ground at the release point. Any subsequent grenades were released sequentially from this identical release point. Figure 2 illustrates the release of a yellow smoke grenade.

Figure 2. Yellow signaling smoke being emitted from a single grenade.

3.1.1 Field Release Events

May — Media were compared for the collection of green signaling smoke and FO during May. Seven green smoke grenades were released sequentially with collection media set 1 m from the release point. For the FO releases, samples were placed on the ground 5 m from the generator release point. Different fogging times were used; 15 min and 18 min of FO release. We also quantified FO deposition in relation to distance from the generation point using jars containing distilled water and heptane. In the May experiments, exposure stations were placed at 5, 50, 100, 500, and 800 m directly downwind of the FO generator release point. Control stations were located 50 m upwind of the release point for all experiments. A set of replicates at each distance was removed following specified time intervals: after 3 and 18 min of fogging during May experiments. After fogging, samples were immediately capped and stored at 4 ºC until extraction and analysis at the Environmental Chemistry Laboratory at ERDC-CERL.

August — Media were compared for the collection of green and yellow signaling smoke during August. For the colored signal smoke releases, samples were placed on the ground 5 or 25 m from the release point. In this case, 20 yellow or green smoke grenades were released sequentially. In the August experiments, exposure stations using jars containing distilled water and heptane were also placed at 5, 25, 50 and 100 m directly downwind of the FO generator release point. A set of replicates at each distance was removed following specified time intervals: after 3, 18, 30, and 60 min during August experiments. Control stations were located 50 m upwind of the release point for all experiments. After fogging, samples were immediately capped and stored at 4 ºC until extraction and analysis at the Environmental Chemistry Laboratory at ERDC-CERL.

3.1.2 Filter Types

Consideration of sampling substrates is critical. For example, FO deposition on bird feathers is measurable (Driver et al. 1999); however, there was no deposition on aluminum foil coupons or glass fiber filters (GFF; Liljegren et al. 1988). This emphasizes the importance of the sample substrate character for collection. Detection on the bird feather likely reveals some adsorptive properties of the feather structure as opposed to the flat, inert surface of typical sample substrates. Sampling substrates will therefore include not only the usual aluminum foil and glass fiber filters, but also filters with different surface chemistries, as well as activated carbon fiber (ACF) filters. Volatile losses from these types of substrates will be minimized due to enhanced adsorptive properties. A comparison of collection efficiencies using these substrates will greatly enhance other field collection studies where volatility losses may have compromised the results.

Table 1 lists all of the collection media that were used in this study. Pall Scientific (Ann Arbor, MI) was chosen as a source of filter media due to the wide variety and availability of their inventory. EKOS Scientific (Champaign, IL, now out of business) supplied the ACF filters.

Table 1. Filters and collection media for deposition of Smokes &Obscurants.

Type of Media	Surface Area (cm^2)	Shape of Media	Manufacturer	Description
Jars of Heptane	44.2	Circle	Sigma-Aldrich®	CHROMASOLV®, for HPLC, ≥99%
Glass Fiber Filters (GFF)	30.25 or 95.03	Circle	Fisher®	Borosilicate glass without binder
Jars of Water	44.2	Circle	DI water	From Millipore filtration system
Foil coupons	30.25 or 80.00	Square	Reynolds®	Aluminum Foil
ICE-450	78.54	Circle	Pall®	Polysulfone with nonwoven polyester support; hydrophilic cationic exchange
Tuffryn	83.00	Square	Pall®	Hydrophilic polyethersulfone
SB6407	78.54	Circle	Pall®	Polyethersulfone copolymer
PTFE	76.00	Square	Pall®	Polytetrafluoroethylene (PTFE) on a polypropylene support
Fiberfilm	85.00	Square	Pall®	Heat resistant borosilicate glass fiber coated with fluorocarbon (TFE)
Emfab	85.00	Square	Pall®	Borosilicate glass microfibers reinforced with woven glass cloth and bonded with PTFE
Nylaflo	85.00	Square	Pall®	Hydrophilic nylon
GHP	80.00	Square	Pall®	Hydrophilic polypropylene
Metricel	80.00	Square	Pall®	Hydrophobic polypropylene
Versapor	78.54	Circle	Pall®	Hydrophilic acrylic copolymer on a nonwoven support
Supor	78.54	Circle	Pall®	Hydrophilic polyethersulfone
FP-450	78.54	Circle	Pall®	Hydrophilic polyvinylidene fluoride (PVDF)

Type of Media	Surface Area (cm^2)	Shape of Media	Manufacturer	Description
ACF-15	30.25	Square	EKOS®	Carbonized and activated phenolic resin coated glass fibers, high surface area
ACF-7	30.25	Square	EKOS®	Carbonized and activated phenolic resin coated glass fibers, low surface area
Basic ACF	30.25	Square	EKOS®	Carbonized and activated phenolic resin coated glass fibers, aminated surface
Oxidized ACF	30.25	Square	EKOS®	Carbonized and activated phenolic resin coated glass fibers, hydroxyl and carboxyl surface

Filters were used as received or were cut to the desired size. The 500-mL jars for solvents were purchased pre-cleaned from I-CHEM (Chase Scientific Glass, Inc., Rockwood, TN) and, when filled with liquid, had a surface area of 44.2 cm^2.

Jars were filled with either water or heptane and were placed at the desired distance from the release point. To prevent the wind from blowing the filters away, they were placed in Petri dishes on the test field as seen in Figure 3. After exposure to S&O, the filters were placed in a 40 mL pre-cleaned I-CHEM vial. The jars of heptane and water were uncapped only prior to and during exposure and were re-capped immediately following exposure. All exposed collection media awaited analysis at a walk-in freezer at ERDC-CERL in Champaign, IL.

Figure 3. A grouping of collection media on the test field at one distance from the release point.

Grabbing an air sample from an S&O cloud will collect all of the components of the cloud, including volatile emissions that will not affect aquatic TES. Clearly, the passive sample arrangement used here allows the analysis of the fraction of S&O that settles from the atmosphere onto a surface to measure actual deposition.

3.1.3 Extractions and Concentrations

3.1.3.1 Filters

Filters exposed to the FO plume during the May and August field experiments were promptly rolled up and placed in I-CHEM 40 mL clear glass vials until extraction. All FO was extracted from the collection media using Sigma-Aldrich Heptane, Chromasolv® for HPLC, ≥99%. A 20 mL B-D Yale glass syringe (Becton, Dickinson & Co., Franklin Lakes, NJ) was used to inject approximately 10 mL of heptane into the 40 mL vial. This initial step was to ensure that any FO vapor contained in the vial was collected by the heptane. The vial was then shaken, and the heptane was collected in a 200 mL Zymark concentrating vessel. The filter was rinsed three to four times with 10–15 mL of heptane, each time combining all heptane into the Zymark concentrating vessel. The contents of the Zymark vessel were then concentrated by a Zymark Turbo Vap II®, which used ultra-high purity nitrogen (S&J Smith, Urbana, IL) to approximately 0.5–1 mL. The resulting concentration was then reconstituted to exactly 2 mL and placed in a gas chromatography/mass spectrometry (GC/MS) vial for automated analysis. Recommended methods for collecting FO samples in the field are documented in U.S. Army Public Works Technical Bulletin (PWTB) 200-01-50.

Filters exposed to colored smoke grenades were extracted using exactly the same method; however, Sigma-Aldrich Dichloromethane, Chromasolv® for HPLC, ≥99.8% was used instead of heptane.

3.1.3.2 Jars

Jars of water exposed to the FO plume were extracted using heptane. The contents of the jar were quantitatively transferred into a 500-mL separation funnel and extracted three times with 20-30 mL of heptane. The jar and lid were also rinsed three times with 5-10 mL of heptane. The extraction solution and rinses were combined and concentrated to a final volume of 0.5-1 mL. The remaining solution was reconstituted to 2 mL using clean heptane and placed in a 2 mL GC/MS vial for analysis.

Jars of water exposed to colored smoke grenades were extracted using exactly the same method. However, Sigma-Aldrich Dichloromethane, Chromasolv® for HPLC, ≥99.8% was used instead of heptane.

Jars of heptane exposed to the FO plume were concentrated down to 2 mL by quantitatively transferring the contents of the jar into three separate Zymark vessels. The jar and lid were rinsed three times with 5-10 mL of heptane and added to the Zymark vessels. As the amount of heptane in each vessel sufficiently decreased, the remaining contents were transferred into one Zymark

vessel. This solution was then allowed to reach approximately 0.5-1 mL, before being reconstituted to 2 mL and placed in a 2 mL GC/MS vial.

Jars of heptane exposed to colored smokes were extracted using exactly the same method as the ones exposed to FO; however, Sigma-Aldrich Dichloromethane, Chromasolv® for HPLC, ≥99.8% was used instead of heptane to rinse the jar and lid (three times), rinse the Zymark vessels as the contents were being transferred, and reconstitute the final solution back to 2 mL.

3.1.4 GC Analysis

Extracts of the above samples were analyzed on either an Agilent 6890 GC/5973 inert MS (GC/MS) with an Agilent 7683 autosampler (Agilent, Wilmington, DE) or a two dimensional GC x GC / flame ionization detector (2D GC/FID) made by LECO Corporation (St. Joseph, MI).

For GC/MS analysis, the FO samples were analyzed using the following parameters:

- An Agilent HP5MS capillary column (30 m x 0.25 mm inside diameter [i.d.] x 0.25 μm film thickness);
- 2 μL splitless injection
- Injection port temperature set at 310 °C
- Oven temperature started at 100 °C for 3 min, ramped at 50 °C / min to 310 °C, and held at 310 °C for 15 min.
- The GC /MS transfer line set at 310 °C.
- The MS mass range set from 35 to 550 amu.

For the May experiments, standards of NFO in heptane were prepared with an injected mass range for NFO from 0.3 to 4 mg. This resulted in a curve with good linearity (R2 =0.98) and the resultant detection limit was approximately 0.1 mg NFO injected. This corresponds to 0.1 mg NFO collected and extracted from a sample jar. For the August experiments, the range for NFO standards was an injected mass from 0.03 mg NFO to 0.4 mg NFO, which again resulted in a curve with good linearity (R2 = 0.98) and a detection limit of approximately 0.01 mg NFO injected (or 0.01 mg NFO collected from a sample jar).

For 2D GC/FID analysis, the FO samples were analyzed using the following parameters:

- Primary column (Phenomenex, Torrance, CA) ZB-1 MS 30 m, 0.25 mm ID, 0.25 μm df
- Secondary column (Restek, Bellefonte, PA) RTX-17 1.1 m, 0.10 mm ID, 0.1 μm df
- Temperature program 1st column — 40 ˚C (0.5 min) – 300 ˚C @ 5 ˚C/min (34 min)
- Temperature program 2nd column — 45 ˚C (0.5 min) – 300 ˚C@ 5 ˚C/min (34 min)
- Modulator offset temperature — 20 ˚C
- Modulation time — 5 sec
- Hot pulse time — 0.8 sec
- Injection — Split / splitless 4 mm open liner
- Temperature — 310 ˚C
- 0.5 μl, split ratio — 100:1

- Flow — 0.8 mL/min constant flow Helium
- Detection — FID 320˚C

For GC/MS analysis, the colored smoke samples were analyzed using the following parameters:

- A Phenomenex 7HG-G002-11 capillary column (5% phenyl, 30 m x 0.25 mm i.d. x 0.25 μm film thickness)
- 2 μL splitless injection
- Injection port temperature set at 250 °C
- Oven temperature started at 50 °C for 3 min, ramped at 20 °C / min to 300 °C, and held at 300 °C for 52 min.
- The GC/MS transfer line set at 310 °C.
- The MS was used in selected ion mode, using 273 atomic mass units (amu) when analyzing for yellow dye and 418 amu when analyzing for green dye.

Graphite flakes settle from the atmosphere as particulate. This particular S&O was not examined further since a simple dish followed by gravimetric analysis will suffice to collect all flakes that will settle onto a water surface.

3.2 Field Toxicity on Aquatic Organisms During Simulated Training Events

3.2.1 Test organisms – Daphnids

Daphnia magna neonates were cultured in the Illinois Natural History Survey Ecotoxicology laboratory according to USEPA (1993) methods using moderately hard reconstituted water (MHRW). Average (± standard deviation) pH, conductivity, alkalinity, and hardness for culture and test water were 8.0 (±0.1), 278 (±6), 62 (±2) mg/L as CaCO3, 86 (±4) mg/L as CaCO3, respectively. Cultures were maintained at constant photoperiod (16L:8D) and temperature (25 ˚C). Before testing, organisms were fed a diet of *Pseudokirchneriella subcapitata* and Yeast-Cereal Leaves-Trout Chow (YCT) mixture daily at rates recommended by USEPA (1993). Daphnia neonates for toxicity testing were acquired using ten 200-mL culture beakers each holding 4 to 5 adult organisms; neonates were removed daily and held in 1 L beakers until they reached appropriate testing age (5-7 d old).

3.2.2 Field Tests

Field experiments testing the toxicity of S&O deposition to aquatic organisms were conducted in spring (May), late summer (August), and winter (December) 2003 at the Aberdeen Proving Grounds. The M56 Coyote Smoke Generating System (SGS) is the U.S. Army's large area smoke generating system. The system was mounted on the back of an M1113 High Mobility Multipurpose Wheeled Vehicle. Military specification fog oil was injected onto a hot manifold and the resultant microdroplets were ejected from the exhaust gas of the SGS to produce a dense white visual obscuration fog. The exit port of the generator was defined as the release point and all distances were measured directly upwind or downwind from this point. The fog oil was injected into the SGS at varying rates adjusted by the operator based on wind speed, direction, and cloud

density. The same system was used for graphite flake dispersion. Graphite flakes were ejected simultaneously with fog oil. Smoke grenades (green, yellow, and red) were released as described above.

Organisms were transported to the field site in 4-L, screw cap containers. On site, organisms were maintained under ambient light and air temperature conditions. In the May experiments, exposure stations were placed at 5, 50, 100, 500, and 800 m directly downwind of the FO generator release point. Following collection and analysis of May data, the stations at 500 and 800 m were eliminated for August exposures, and a 25 m station was added. Control stations were located 50 m upwind of the release point for all experiments. A set of four replicate test chambers containing organisms was placed at each exposure distance for each fogging duration. Each test chamber was a 450-mL glass I-Chem® jar (Thermo Fisher Scientific, Pittsburgh, PA) filled with 300 mL of MHRW and loaded with 5- to 7-day old *D. magna* neonates (n=5 per jar). These jars have a surface opening of 44.2 cm^2 available for deposition, a surface to volume ratio of 0.147.

Measured water temperatures in test chambers throughout the testing period ranged from 15–16 °C in May and 20–22 °C in August. The warmer August air temperatures required a cooling mechanism to keep test waters in the appropriate temperature range (20–22 °C). This was accomplished by nesting jars into trays of ice during field exposures. A set of replicates from each distance was removed following specified time intervals: after 3 and 18 min of fogging during May experiments, and after 3, 18, 30, and 60 min during August experiments. Following exposure, jars were immediately re-capped and transported to a mobile laboratory where mortality and floaters were recorded at 24 and 48 h post-exposure. Note that organisms caught in surface film at 24 h may not necessarily be caught in surface film at 48 h, therefore it was possible to have lower numbers for % floaters at 48 h than at 24 h. Jars containing distilled water and heptane were also placed at each exposure station to quantify oil deposition. After fogging, these samples were immediately capped and stored at 4 °C until extraction and analysis.

3.2.3 Statistical Analysis

Lethal concentrations for 50% of the tested organisms (LC_{50}) were calculated using the Spearman-Karber method, or probit analysis (Hamilton et al. 1977). For field testing, mortality data were analyzed using Toxstat 3.5 and JMP IN software (Sall and Lehman, 1996). Differences between treatments in mean percent mortality were determined using Fisher's Exact Test (p=0.05). Percent of floater data were tested for normal distribution (Shapiro-Wilks test) and for homogeneity of variance (Hartley's and Bartlett's tests). Non-normal data with the same number of replicates were analyzed using Steel's Many-One Rank Test. Non-normal data with different number of replicates were analyzed in JMP IN by Wilcoxon/Kruskal-Wallis Tests (Rank Sums).

3.2.4 Other Test Organisms

3.2.4.1 Fauna

Midge *(Chironomus tentans)* individual and mass cultures were maintained in the INHS ecotoxicology laboratory according to USEPA (1994) methods at constant photoperiod (16 hours light : 8 hours dark) and temperature (22 ˚C). Test waters were the same as described above. Prior to

testing, organisms were fed a mixture of 20 g TetraMin® flake food and 20 g Kaytee® Forti-Diet ® rabbit food (antibiotic-free) per 1 L of deionized water. Individual cultures were comprised of three to five egg cases and were held in 767 mL Rubbermaid® containers. Each was fed at a daily rate of 3 mL mixture per 400 mL water. Aeration and feeding began upon hatching. Midges were 18-20 d old upon initiation of testing. Fathead minnow (*Pimephales promelas*) fry and adults, and northern leopard frog (*Rana pipiens*) larvae, and were obtained from standard commercial sources. Fountain darter (*Etheostoma fonticola*) fry and adults, and Topeka shiner (*Notropis topeka*) adults were obtained from the U.S. Fish and Wildlife Service and the Missouri Department of Conservation respectively under Endangered Species Act Recovery Permit authority. Rainbow trout (*Oncorhynchus mykiss*) juveniles were obtained from the Colorado Division of Wildlife. Fish and frogs were generally shipped to ERDC-CERL. In some instances, fountain darters were shipped directly to APG/Army test site. Frog larvae were maintained on standard flake food (as described above). Fish were provided brine shrimp (*Artemia salina*) nauplii hatched at the ERDC-CERL or Army test site. The physical parameters of the water for the fish and frogs are described above.

3.2.4.2 Flora

Stuckenia pectinatus plants were purchased as tubers from Wildlife Nurseries, Oshkosh, WI. Tubers were germinated in topsoil either in greenhouse aquaria (May) or outdoor stock tanks (August) two weeks prior to transport to the APG/Army test site. Plants were segregated in pots within the aquaria and stock tanks. One day prior to transport, the tubers and new underground rhizomes were clipped off the plants to insure uniformity in sample morphology. The plants were approximately 10 – 15 cm long at the time of transport. For transport, the roots of the plants were pushed into a 6-cm-deep layer of cat litter in large plastic vats. The vats were half-filled with well water prior to planting and sealed with plastic lids.

Just prior to exposure, the plants were transferred from the holding vats to standard 500 mL screw cap glass jars (Thermo Fisher Scientific, Pittsburgh, PA) containing approximately 350 mL moderately hard reconstituted synthetic (MHRS) water (EPA 2002). Two plants were placed in each jar, and maintained at ambient temperature. Jars were kept capped prior to exposure. Four replicate jars were placed at each exposure distance for each exposure time.

After exposure, the jars were capped and stored in the dark for 24 h. At 24 h post-exposure, the plants were placed, along with some of the exposed water, into plastic bags and sent by overnight express to Purdue University for analysis. Immediately upon receipt, the plants were removed from the water. The total time of immersion in exposed water was approximately 48 h.

From each jar, the leaves of one plant were excised, and a 7 cm leaf segment was analyzed for chlorophyll (mg chl a/g FW) using the dimethylsulfoxide (DMSO) extraction method of Hiscox and Israelstam (1979). The other plant was planted into an outdoor stock tank to determine the amount of new growth, as measured by total stem length (cm) and biomass (g dry weight [DW]). Measurements were taken on each plant before and after a 3 week period. Loss of chlorophyll was used as an indicator of impaired physiology and reduced productive capacity. Changes in biomass were used as an indicator of plant growth (Van Wijk 1988; Lehmann et al. 1994; Best

and Boyd 2003). These data were used to provide information on the potential of FO obscurant to cause developmental abnormalities during growth.

Green algae (*Pseudokirchneriella subcapitata*) (old name *Selenastrum capricornutum*) was originally obtained from the UTEX Algal Culture Collection (No. 1648). Five days prior to travel, the alga was inoculated in 6 L flasks and grown in MHRS water with nitrogen and phosphorus additions. The inoculated flasks were incubated in a controlled environment growth chamber at 25 ºC, 120 µmol photons/m²/s, 16:8 hours light:dark, and with constant aeration (ambient air). The morning of travel, the flasks were poured into plastic carboys for transport. Just prior to exposure, 50 mL of algal solution was added to 300 mL of MHRS water in 500 mL glass jars, and maintained at ambient temperature. The jars were kept capped until exposure. Four replicate jars were placed at each exposure distance for each exposure time.

After exposure, the jars were capped and stored on site in the dark for 48 hours. One hundred mL of each jar's contents were vacuum pumped through 4.7 cm glass fiber filters. The filters were immediately placed on ice packs and sent by overnight express to Purdue University. Upon receipt, the filters were stored in a freezer until analysis for chlorophyll. Chlorophyll (µg chl a/L) was analyzed using the modified dimethylsulfoxide method of extraction (Burnison 1980) and the equations of Lorenzen (1967). Loss of chlorophyll was used as an indicator of contact damage and impaired physiology (Abou-Waly et al. 1991; Van Der Heever and Grobbelaar 1996; Beardall et al. 2001). *Pseudokirchneriella subcapitata* was used in the August field exposure only.

Exposure distances from the fog release point and exposure times are defined in the data tables below. Plant data were subjected to analysis of variance (ANOVA) procedures using SAS (SAS Institute 1988). A Dunnett's test was used to determine differences of the exposed means from the control mean.

Prior to exposure, 2 – 5 individuals of each test organism were placed into test jars and transported to the exposure stations. Four replicates for each species were placed at each exposure distance. Jar lids were removed immediately prior to S&O release and capped and transported to an onsite mobile laboratory immediately following exposure. Tested exposure durations ranged from 1 – 14 min for colored smoke grenades and 3 – 120 min for fog oil and fog oil plus graphite. Test animals were observed for a minimum of 48 hours, and mortality was evaluated at the end of the test period. Test animal observation was done at the field test site, in transit from the test site, and at INHS and ERDC-CERL laboratories.

Relevant life stages of test organisms were exposed to various concentrations of S&O deposition (as determined by distance from release point) of green, yellow, and red signal smokes, fog oil smoke, and fog oil and graphite combination smoke (Tables 2, 3, and 4). Exposures were done during May, August, and December to coincide with typical spring, summer, and winter field S&O release conditions. Based on experience gained and following data collection and analysis of the May field exposures, to be more relevant, distances were generally reduced and exposure times generally increased during the August and December field exposures.

Table 2. Summary of *D. magna, C. tentans, P. promelas,* and *R. pipiens* exposures to smokes and obscurants (May). C = Control, 50 m upwind.

Smoke or Obscurant	# Grenades or Duration	Exposure distance (meters)
Red Smoke	1	C, 5, 25, 50, 100, 250, 500, 800
Red Smoke	6	C, 5, 25, 50, 100, 250, 500, 800
Green Smoke	1	C, 5, 25, 50, 100, 250, 500, 800
Green Smoke	7	C, 1, 5, 25, 50, 100, 250, 500, 800
Yellow Smoke	1	C, 5, 25, 50, 100, 250, 500, 800
Yellow Smoke	7	C, 5, 25, 50, 100, 250, 500, 800
Yellow Smoke	16	C, 1
Fog Oil Obscurant	3 min	C, 5, 25, 50, 100, 250, 500, 800
Fog Oil Obscurant	18 min	C, 5, 25, 50, 100, 250, 500, 800
Fog Oil & Graphite	13:35 min	C, 50

Table 3. Summary of *D. magna, E. fonticola,* and *R. pipien*s exposures to smokes and obscurants (August). C = Control, 50 m upwind.

Smoke or Obscurant	# Grenades or Duration	Exposure distance (meters)
Red Smoke	20	C, 5, 25
Yellow Smoke	20	C, 5, 25
Green Smoke	20	C, 5, 25
Fog Oil Obscurant	3 min	C, 5, 25, 50, 100, 250
Fog Oil Obscurant	18 min	C, 5, 25, 50, 100, 250, 500
Fog Oil Obscurant	30 min	C, 5, 25, 50, 100, 250, 500, 800
Fog Oil Obscurant	60 min	C, 5, 25, 50, 100, 250, 500, 800
Fog Oil Obscurant	120* min	C, 5, 25, 50, 100, 250, 500, 800
Fog Oil & Graphite	3 min	C, 5, 25, 50, 100, 250
Fog Oil & Graphite	18 min	C, 5, 25, 50, 100, 250, 500, 800
Fog Oil & Graphite	60 min	C, 5, 25, 50, 250, 500
Fog Oil & Graphite	120* min	C, 5, 25, 50, 100, 250, 500

* nominal time

Table 4. Summary of *N. topeka* and *O. mykiss* exposures to smokes and obscurants (December). C = Control, 50 m upwind.

Smoke or Obscurant	# Grenades or Duration	Exposure distance (meters)
Red Smoke	19	C, 1, 5
Green Smoke	22	C, 5, 25
Yellow Smoke	20	C, 1, 5
Fog Oil Obscurant	18 min	C, 5, 25, 50, 100
Fog Oil Obscurant	30 min	C, 5, 25, 50, 100
Fog Oil Obscurant	60 min	C, 5, 25, 50, 100
Fog Oil Obscurant	120* min	C, 5, 25, 50, 100
Fog Oil & Graphite	18 min	C, 5, 25, 50, 100
Fog Oil & Graphite	30 min	C, 5, 25, 50, 100
Fog Oil & Graphite	60 min	C, 5, 25, 50, 100
Fog Oil & Graphite	120* min	C, 5, 25, 50, 100

* nominal time

3.3 Laboratory Testing of Fog Oil Toxicity

These experiments involved both exposure of organisms to fogging within an enclosed chamber and injection of oil at the water surface. All fogging experiments were performed with HOC FO, a new fog oil and also the same oil used in the field experiments.

3.3.1 Chamber Release

Due to the difficulties of field testing and control of environmental conditions, an experiment was also performed within an enclosed chamber constructed at ERDC-CERL specifically for S&O release. The environmental chamber at ERDC-CERL is a 49.9 m^3 (2.65 m H x 4.05 m W x 4.65 m L) enclosure used for conducting experiments with military S&O to simulate field conditions under controlled conditions. Figure 4 shows the front wall of the chamber. The walls, ceiling, and doors were constructed with panels from U.S. Cooler (Quincy, IL) with smooth stainless steel interior surfaces. The floor was constructed in-house from plywood and spray foam insulated from the underside. The cooling system for the chamber is also from U.S. Cooler. The small door to the lower left of the picture reveals a smaller internal chamber (1 m H x 0.92 m W x 0.92 m L) that can be used for controlled releases of S&O. The small internal chamber has its own controllable damper system so that a user can release smoke into the large chamber or vent it out through an external filtration unit.

The larger chamber is equipped with a wall vent and fan that pull air into the chamber from outside the building, and a ceiling vent and fan that pull air out of the chamber and release it outside the building. This flow system is used for evacuation of smoke from within the chamber. Both vents can be opened and closed using dampers that, in addition to the fans, are controlled from outside the chamber. A third vent in the side wall can be opened to create a passageway between the chamber and the outside of the building.

Figure 4. Exterior of the environmental chamber.

Inside the chamber are 24 sets of ultraviolet (UV) lights separated into four banks that can be used to simulate different levels of UV radiation from the sun depending on how many banks are turned on. The temperature of the chamber is controlled with a radiator for heating and a built-in air-conditioning system for cooling. This arrangement allows for simulation of outside environmental conditions from winter to summertime conditions. Two real-time aerosol sensors (Model RAS-2 from Monitoring Instruments for the Environment, Inc.) are used to monitor the optical density of the smoke within the chamber during experiments. One is located approximately 1 ft off the floor, and the other is approximately 7 ft off the floor directly above the first sensor. The sensors are connected to an Omega data logger (Model 0M550) outside the chamber that is directly interfaced to a laptop. The computer program DataWorker LE for Windows (Omega Engineering, Inc., Stamford, CT) downloads and displays the sensor readings.

3.3.2 Environmental Chamber Protocol

The first step in using the environmental chamber is to set the internal chamber temperature. A temperature of 25 °C was used for the experiments below. A thermometer in the interior of the chamber lets the user know when the chamber has reached the desired temperature. A clean piece of aluminum foil is placed on the floor of the chamber. The samples to be tested are placed on the foil. The computer and the sensor data logger can be started at this point. Once the chamber has reached the desired temperature, the main door of the chamber is closed and rope insulation and duct tape are applied around the door to minimize leakage. The interior floor fan is turned on at this point to ensure mixing of fog throughout the chamber.

The fog generator (Figure 5) is moved outside of the building for use during experiments. A MasterFlex pump is connected to the generator to deliver HOC FO to the generator. As the generator warms up, the sensors can be tested to ensure they are receiving data from inside the chamber. Once the generator temperature reaches 450 °C, the oil pump is turned on. The chamber ceiling damper is opened and the ceiling fan is turned on to pull the fog into the chamber. When the generator is producing a steady stream of fog, an intake pipe is placed in front of the generator exhaust pipe for the desired fogging time for the experiment. In this experiment, the chamber was fogged with HOC FO for 2 min. The intake pipe directs the fog into the chamber through a vent in the wall of the chamber. After 2 min, the intake pipe is blocked to prevent further introduction of fog, the ceiling fan is turned off, and the ceiling damper is closed as quickly as possible. The oil pump is stopped and the generator is turned off to cool. The fog remains in the chamber for a set residence time. Once this time has expired, the ceiling and wall dampers are opened and their fans turned on to evacuate the chamber of fog. Readings from the aerosol sensors are used to determine when the chamber is evacuated to baseline levels. In this experiment, all of the fog within the chamber was allowed to settle for 9 h.

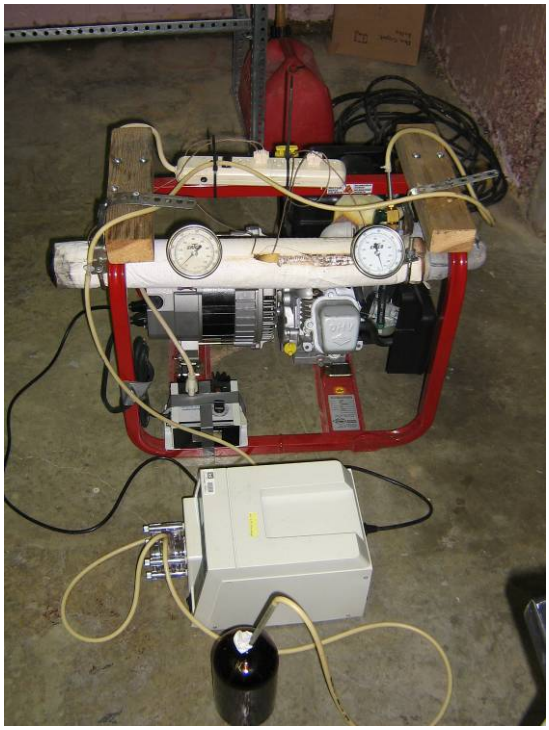

Figure 5. Fog generator and oil pump.

3.3.3 Chamber exposure protocol

All chamber exposures were conducted in 500-mL, I-Chem glass screw cap jars with Teflon lid liners, with MHRW as a test medium (USEPA 1993). The protocol followed was generally as follows. Prior to exposure, 2 - 5 individuals of *Daphnia magna* were placed into test jars which were then placed on the floor of the fogging chamber. Four replicate jars are used for each fogging experiment. Test animals were observed for a minimum of 48 hours, and mortality was

evaluated at the end of the test period. Four replicate jars of each test organism are also used as controls that are kept outside of the chamber.

3.3.4 Fog oil injections

Laboratory experiments were conducted in order to better understand if mortality is due to exposure to the surface oil film or suspended oil droplets or if it is due to exposure to dissolved FO components. One type of test was designed to allow the neonates exposure to a FO film that was injected onto the water surface to simulate deposition of FO (surface injection tests). Another type of test allowed neonates standard swimming room but prevented exposure to the injected FO surface film (separation tests). The separation tests allowed exposure to FO components in the aqueous phase and prevented the floater phenomenon from occurring.

Past chemical composition studies showed that older stocks of fog oil contained about 50% aromatic hydrocarbons (Katz et al., 1980). In 1986, new military specifications for fog oil required the purchases of stocks where naphthenic oils had been further refined through hydro-treatment and solvent extraction in an attempt to drastically reduce or eliminate the aromatic compound fraction (NRC et al., 1997). Unrefined fog oils produced before 1986 are referred to as old fog oils while those produced according to the changed military specifications are referred to as new fog oils. While field experiments were conducted using only new HOC FO, these laboratory experiments investigated HOC FO as well as two older types of FO, one obtained from Ft. Irwin, made in 1982, that we denote as OFO1, and one obtained from AMCO, made in 1981, that we denote as OFO2. The relative toxicities of these three types of FO were compared.

For comparison of initial versus generated fog oil, we collected a sample of the NFO oil fog as it condensed onto a cold surface directly placed in front of the exhaust port. Surface-injection LC_{50} tests were conducted as described above for initial oils, but at higher oil concentrations.

Ceriodaphnia dubia (Figure 6), a typically more sensitive cladoceran commonly used in freshwater toxicity tests, was cultured according to USEPA methods (1993). Twenty-four-hour-old *C. dubia* neonates were loaded into 50-ml beakers filled with 40 mL of MRHR (USEPA 1993) water using a Finnpipette pipettor or a graduated cylinder.

Figure 6. ***Ceriodaphnia dubia.***

In the surface injection experiments, oil was injected onto the water surface at 6 treatment concentrations: 0.0 µL total oil / 40 mL water, 0.1 µL total oil/ 40 mL water, 0.5 µL total oil/ 40 mL water, 1.0 µL total oil/ 40 mL water, 5.0 µL total oil/ 40 mL water, and 10 µL total oil/ 40 mL water. Using an experimentally derived density of FO equal to 0.9 g/ mL, these treatment concentra-

tions correspond to 0 mg FO/ L water, 2.25 mg FO/ L water, 11.27 mg FO/ L water, 22.5 mg FO/ L water, 112.7 mg FO/ L water, and 225 mg FO/ L water. Four replicate beakers were used per treatment, and each beaker was loaded with 5 neonates prior to adding oil (Figure 7). A 10 µL syringe or a Finnpipette micro pipettor was used to apply the oil.

In the separation tests, oil was added by surface injection; however, a separation apparatus was used to discourage neonate contact with the surface oil film (Figure 8). Thirty-ml glass jars with polyethylene screw caps were used as the allotted swimming space for the 5 neonates. The top of each cap was removed and the threaded portion was used to fasten a 105 µm nylon mesh as the new lid to the jar once neonates had been loaded. These jars were each nested inside of a 150-ml beaker, the remaining test water was added (total water = 90ml) creating approximately 1.5 cm of headroom between the water surface and the mesh cap.

Figure 7. A dose response curve surface injection experiment.

Figure 8. Glassware used in dose response curve / separation experiments.

This allowed exchange of water and potential water-soluble FO components in and out of the allotted swimming area for the neonates while also discouraging neonate contact with the surface oil film. Six treatments (4 replicate beakers per treatment) including a control with no added oil were tested. Range-finding test were conducted using concentrations from 0.225 µL total oil/ 90 mL water (2.25 mg/ L) to 22.5 µL total oil/90 mL water (225 mg/ L). Subsequent testing was also conducted at 375 µL total oil/ 90 mL water (3756 mg/ L) and 500 µL total oil/ 90 mL water (5007 mg/ L).

During the 48-hour observation period, each treatment was covered in a separate piece of plastic wrap to prevent evaporation and transference of oil. Mortality and floaters were monitored at 24- and 48-hours during surface injection tests. Mortality alone was monitored in separation tests. Each test type was replicated 3 times to increase confidence in results.

During a one surface injection test, the water-soluble fraction of the exposure water was collected at 48 h by using a 10-ml syringe to draw subsurface water through a borosilicate glass pipette that was placed into each beaker prior to addition of FO surface injection. The initial 1-2 mL of drawn water was discarded and 7-10 mL were kept refrigerated until quantification and

compositional analysis using two dimensional gas chromatography with flame ionization detection (GC x GC FID).

3.3.5 Statistical Analysis

LC_{50} values were calculated using Spearman-Karber method, or probit analysis ($p<0.05$). Mean LC_{50} generated from the 3 replicate tests for the surface and injection and for the separation tests were compared using a one-way ANOVA ($p < 0.05$) and a student's t-test for pair-wise comparisons. Because raw data (percent mortality, not LC_{50}s) were not normally distributed and had unequal variance, we performed a ranked 2-way ANOVA with oil type and oil concentrations as variables affecting mortality. This additional ANOVA looked for significant effects between several combinations of oil type and oil concentration. Means separation was used to compare oil type for each of the various oil concentrations. There was a high probability that a significant difference would be encountered in the 18 combinations that were tested; therefore, a Bonferroni adjustment was used ($p < 0.009$).

3.4 Laboratory Midge Experiments

Test chambers were held in an environmental growth chamber at 23°C at 16:8 light/ dark. Test chambers comprised of 1000 mL glass beakers containing 500 mL MHRW. As habitat for the *Chironomus dilutus* larvae, each beaker contained three 2-ply strips of 4 x 9 cm unbleached paper towels which were boiled twice to remove impurities. Towel strips were stacked together and bound centrally with a plastic zip tie and placed on the bottom of the beakers.

Each beaker was fitted with two 24-cm long pieces of aquarium tubing secured to the inside of the beaker with lab tape so that one end of each tube extended over the lip of the beaker and the other end rested approximately 4 cm from the bottom of the beaker. The tubes were attached to a Masterflex Console Drive peristaltic pump used to pump fresh water in and out of the beaker at the same rate of speed to minimize disturbing the surface layer of injected oil. 1500 mL of overlying water was removed and replaced using this method twice a day. The water outtake tube of each beaker was fitted with a piece of nylon mesh as a screen to prevent pupae or larvae from being pumped out of the beaker during water changes. Dissolved oxygen was tested on water pumped from each beaker daily. Treatments consisted of the following: a control (MHRW), 1 µL surface injected generated fog oil / 100 µL surface injected generated fog oil. Fog oil was generated at ERDC-CERL as described previously. Ten *C. dilutus* larvae were placed in each replicate beaker. In June, there were five replicates per treatment and in February, there were four replicates per treatment.

C. dilutus were fed 1 mL of a slurry of 5 g TetraMin in 1 L MHRW immediately after the first daily water change. Food was introduced through the fresh water intake tube of each beaker using a pipette. A small amount of MHRW was pumped through the intake tube to flush the slurry into the beaker.

Larvae were added to beakers and allowed to acclimate for 24 h before fog oil was injected on the surface of the treatment replicates using pipettes. Beakers were checked daily for larval death, emergence of pupae, and adult emergence and sex. Partial emergence of adults and com-

pletely emerged adults, who could not break free from the surface tension of the water, was also recorded.

3.5 Laboratory Assessment of Photolyzed Fog Oil Toxicity

The non-standardized FO used in the various toxicity trials during this portion of the project was converted to smoke; converted to smoke then condensed back to a liquid before agitating with water; and exposed to sunlight while layered on water. The FO used met the 1986 military specification that limited the amount of aromatic compounds present (NRC et al. 1997). Fog oil smoke was generated by injecting oil into a manifold heated to 350 ºC. The resulting vaporized FO was released for a timed period into a 12' x 12' x 8' chamber in which 450 mL glass I-Chem® beakers (Thermo Fisher Scientific, Pittsburgh, PA) filled with water (synthetic moderately hard water (SMR water), pH 7.8, hardness 92 mg CaCO3/L, conductivity 305 µS/cm2, alkalinity 66 mg CaCO3/L; USEPA, 2002) and test organisms were placed on the floor. The aerosolized oil was allowed to settle onto the water surface of the beakers holding the test organisms for a second timed period. The chamber's exhaust fan was used to evacuate the smoke at the end of the exposure. It was determined during preliminary testing that production of smoke for 120 second and the retention of that smoke for 9 h was equivalent to a typical simulated battle field training exposure (unpublished data, Cropek).

To obtain FO smoke combined with water, a cold (ambient temperature 0 °C) aluminum surface was placed 0.3 m from the manifold during smoke production. The heated oil that condensed on the aluminum surface was collected. To eliminate the surface film problems associated with the toxicity testing of a poorly water soluble oil, the post-manifold FO was subjected to a modified water accommodated fraction (WAF) sample preparation technique (Maher 2005). Instead of stirring the post-manifold oil into the SMH water through low vortex energy as recommended by Maher (2005), the oil and water were violently agitated together in a paint mixer (Red Devil Auto Sperse, Plymouth, MN 55447). The 1-quart paint mixer was used to individually prepare the 300 mL of SMH water plus post-manifold oil mixtures. Each mixture was shaken for 5 min. Replenishment mixtures for the static run trails were prepared daily and were used to replace 80% of the original test volume as called for in EPA Whole Effluent Toxicity (WET) Methods when determining a 96-h LC_{50}.

To obtain the sunlight exposed FO, 10 mL of FO was added to 10 L of SMH water in an uncovered 75-L aquarium. The FO and water were then exposed to direct sunlight for 4 d. Three batches were produced and analyzed for Total Organic Carbon (TOC) and pH. TOC values were 38, 40 and 48 ppm, and pH values were 7.0, 7.8, and 7.0. A siphon was used to remove photo-oxidized FO water without disturbing the floating FO layer above.

3.5.1 Test Fish Production and Shipping

Fountain darters utilized in these tests were produced at the NFHTC and were hatchery reared offspring from wild adults collected from the San Marcos River, Hays County, and the Comal River, Comal County, in central Texas. Fish were cultured in chilled Edwards Aquifer ground water (EAG water; temperature 19 °C± 2, alkalinity 319 mg/L and hardness 300 mg/L as CaCO3). Spawning was coordinated to produce eggs, larvae, and juveniles of precise and consis-

tent ages for the tests. The eggs were 24 to 48-hour post-fertilization to accommodate the delay of overnight shipment. Age of larvae used was 2 to 4-days post-hatch (total lengths of 3.8-4.4 mm). Fountain darters initially start feeding when they are 2 to 4 d old and are at a similar physiological stage as <24-hour old fathead minnows are when they are routinely used in toxicological test. Also, at this stage larvae show improved resilience to handling. Juveniles were 30-days post-hatch, the same age dictated by USEPA and ASTM guidelines for fathead minnows. Adult breeders were 2-years old with standard lengths of 24-32 mm.

All exposure of the fountain darters to FO was done at the ERDC-CERL. Eggs, juvenile, and adult fish were shipped by commercial overnight delivery (FedEx) in plastic bags in ice chests to ERDC-CERL from the NFHTC. Water temperature was maintained between 16-21 °C during shipment. Frozen gel packs were added to ice chests during warm weather. Each adult male/female pair was shipped with 400 mL of EAG water in a 1-quart Ziploc ® freezer bag inflated with oxygen. Eggs and juveniles were bulk shipped in larger plastic bags containing ~3 L of water and inflated to a ~9-L volume with oxygen. Larvae were shipped as eggs and allowed to hatch in aquaria at ERDC-CERL to minimize shipping mortality.

3.5.2 Toxicity of FO Smoke to Adults, Eggs, and Larvae

Adult, eggs, and larvae fountain darters were exposed to FO as smoke to test its toxicity. Fountain darter adults were shipped in EAG water from NFHTC to ERDC-CERL. The treatment group consisted of 24 male and female pairs. Each pair was placed in individual beakers with 300 mL of SMH water in the chamber and received a 120-second fogging/ 9-hour deposition dose. A second group of 24 breeding pairs were in 24 beakers that were not exposed to FO smoke (controls). At the end of the smoke exposure, each pair of treated and control fish were placed back into their original Ziploc® shipping bags containing 1/3 EAG water and 2/3 oxygen by volume. The fish were shipped overnight back to the NFHTC. Arrival back in Texas completed a roundtrip of ~ 3 days.

Two breeding pairs were then randomly stocked in each of 24 7-L glass aquaria; 12 aquaria contained treated fish and 12 contained control fish. Each aquarium contained a 10-cm length of 7.6-cm PVC pipe cut lengthwise to be used as spawning substrate by the fish. The PVC spawning substrates were removed and replaced on days 5, 9, 13, 17, and 21. Eggs present on a spawning substrate and the sides of an aquarium were counted and then incubated in a separate adjacent aquarium (a total of 24 incubation aquaria). After the eggs were removed from an aquarium, a siphon tube was used to remove waste from the aquarium bottom. Before additional eggs were added to an incubation aquarium on days 9, 13, 17, and 21, all eggs within an incubation aquarium were inspected and the non-viable eggs were counted and discarded. Any larvae present also were counted and removed. Eight days after the last eggs were moved into an incubation aquaria, the numbers of viable eggs, non-viable eggs, and larvae present in each aquarium was determined.

The 48 total aquaria (24 for adult fish and 24 for incubating eggs) were placed on top of three 530-L insulate fiberglass reservoir tanks (Living Stream, Frigid Units Inc. Toledo, OH, USA) equipped with 0.5-hp pumps (Hayward Industries, Elizabeth, NJ, USA) and heater/chiller units (Universal Marine Industries, Anmore, BC, Canada) which maintained water temperature at 21.4

± 0.3 ºC and total gas saturation below 94%. Water was exchanged in each aquarium every 0.5 h and EAG water was added to the reservoir at the rate of ~ 1 L/min. During the spawning period, the fish were fed daily live blackworms (*Lumbriculus variegatus*). Standard lengths of the adult fish were determined at the end of the spawning period. No adult mortalities occurred during the shipping, exposure, return shipping and spawning period.

Fountain darter eggs that were <24 h old were overnight shipped in EAG water from NFHTC to ERDC-CERL. Upon arrival at ERDC-CERL, the eggs were individually inspected for viability. Thirty clear eggs were placed in each of four beakers with 300 mL of SMH water and exposed daily for three consecutive days to 120 seconds of FO smoke production and 9 h of smoke retention. Two beakers containing 30 eggs each and SMH water and two beakers containing 30 eggs each and EAG water were not exposed to the FO smoke. Fungused eggs were removed daily and on days 5 and 8 post initiation of trial, each beaker was inspected and the numbers of viable eggs, non-viable eggs, and larvae were recorded. Dissolved oxygen, pH, and temperature were measured daily in three randomly chosen smoke exposed beakers and in all control beakers. Initial and final alkalinity, hardness, and conductivity were measured in three randomly chosen beakers. A second batch of eggs was shipped to ERDC-CERL and a replicate trial was conducted.

A portion of the eggs shipped were allowed to hatch at ERDC-CERL. Ten 2- to 4-day old larvae were each placed in 10 beakers containing 300 mL of SMH water. The larvae were exposed to 120 seconds of FO smoke production and 9 h of smoke retention daily for 7 d. Ten beakers each containing 10 larvae and EAG water and ten beakers containing 10 larvae and SMH water were not exposed to the FO smoke. Larvae were fed live brine shrimp (*Artemia salina*) daily following modified USEPA (2002) procedures. The brine shrimp were washed several extra times to limit fountain darter mortality associated with ingestion of un-hatched brine shrimp eggs. Dissolved oxygen, pH, temperature, alkalinity, hardness, and conductivity were measured following procedures described earlier. The number of alive and dead larvae in each beaker was recorded daily through day 8. A second group of larvae hatched at ERDC-CERL were used to conduct a replicate trail.

3.5.3 Toxicity of FO Smoke WAF to Eggs and Larvae

Fountain darter eggs and larvae were used to determine the toxicity of the FO smoke WAF. Thirty clear eggs were placed in each of four beakers with 300 mL of each of the post-manifold oil/SMH water mixture test concentrations. A range finding trial was conducted to determine the final testing concentrations of 0, 900, 1275, 1650, 2025, and 2400 mg/L of post-manifold oil. The eggs in each beaker were inspected daily and the non-viable ones were removed. At the end of 96 h, the number of viable eggs was recorded. Water quality was measured as described earlier.

Ten 24- to 48-h old larvae were placed in each of four beakers with 300 mL of each of the post-manifold oil/SMH water mixture test concentrations. The concentrations tested included 0, 150, 300, 600, 1200, and 2400 mg/L. The larvae were fed brine shrimp daily. Mortalities were removed daily and number of surviving larvae in each beaker at the end of 96 h was determined. Water quality was measured as described for earlier trials.

3.5.4 Generated Fog Oil UV Exposure Tests

Thirty 150 mL beakers are fitted with a tubing apparatus that allows collection of water samples from the bottom of the beaker. Twenty-five milliliters of DI water is added to each beaker. 0.3 mL generated fog oil is deposited on the surface of the water using a pipettor. All beakers are weighed individually and their weights recorded. Half of the beakers are placed in the environmental chamber and exposed to all 4 banks of UV lights. The other half are controls which are kept in the dark. After 1 day, three control beakers and three treatment beakers are weighed and the water lost by evaporation is replaced by using a needle and syringe to penetrate the film of oil on the surface of the water. Water samples are then taken from all six beakers. 10 mL were analyzed for total organic carbon content to determine the increase in oil components in the water column. This is repeated on Days 2, 3, 7, and 14.

3.5.5 Toxicity of Sunlight-Exposed FO to Eggs, Larvae, and Juveniles

Static renewal trials were used to determine sunlight exposed FO 96-h LC_{50} values for fountain darter eggs, larvae, and juveniles. Each trial was run with five photo-oxidized FO dilutions, a SMH water control, and four replicates per dilution. Dilutions were chosen after preliminary range finding trials were completed. Test solutions for 80% daily replenishments were prepared immediately prior to solution renewal. Final dilutions used were: eggs- 0, 10, 15, 25, 35, and 45%; larvae- 0, 11, 13, 15, 17, and 19%; and juveniles- 0, 11, 13, 15, 17, and 19. Each replicate received 300 mL of test solution and either 30 eggs, 10 larvae, or 10 juveniles. Eggs, larvae, and juveniles were inspected daily and mortalities counted and removed. Larvae and juveniles were fed brine shrimp daily. Water quality was measured as described for earlier trials.

3.5.6 Toxicity Tests with Photolyzed Water on C. dubia

Forty-eight 150 mL beakers are fitted with tubing apparatus that allows collection of water samples from the bottom of the beaker. Twenty-five milliliters of EPA water is added to each beaker. 0.3 mL generated fog oil is deposited on the surface of the water of forty of the beakers using a pipettor. The other eight will be used as controls. All beakers are weighed individually and their weights recorded. Then they are placed in the environmental chamber and the lights turned on. After 1 day, eight beakers with oil are weighed and the water lost by evaporation is replaced with DI water using a needle and syringe to penetrate the film of oil on the surface of the water. Water samples are then taken from all eight beakers and combined. This is repeated on Days 2, 3, 7 and 14. On Day 14, the controls are taken down as well. It is necessary to replenish the water in the controls on almost a daily basis due to the rate of evaporation.

3.5.7 Statistical Analyses

The effects of smoke exposure on adults were evaluated in a repeated measures one-way analysis of variance (ANOVA) design. The response data of egg output and viability were analyzed using JMP-In®(SAS Institute, Belmont, CA, USA) with no transformations. Statistical significance throughout the experiments was assumed at $p < 0.05$.

Response data of eggs, larvae, and juveniles exposed to WAF mixtures and dilutions of photo-oxidized FO were evaluated with trimmed Spearman-Karber tests. This nonparametric procedure run on EPA-provided software calculated chronic mortality indices (LC_{50} and 95% confidence interval [CI]).

3.6 Analysis of Fog Oil

Several standards were used in this study to place hydrocarbon classes within the separation plane. A standard mixture of n-alkanes from C9 to C36 (Restek, Bellefonte, PA, Cat. No. 31459) was used to note the position of these paraffins as well as identification of the carbon numbers within FO. Two other pre-mixed standards, one containing the highly branched hydrocarbons pristine and phytane (Restek, Cat. No. 31240), and one containing polynuclear aromatic hydrocarbons including anthracene, benz[a]anthracene, benzo[a]pyrene, benzo[e]pyrene, chrysene, fluoranthene, perylene, phenanthrene, pyrene, and triphenylene (Supelco, Bellefonte, PA, Cat. No. 4-9155) provided retention data for comparative purposes. Mixtures of individual compounds were also employed as needed. Branched cyclohexanes, and long chain alkenes and alkynes identified the location of these broad classifications. These include octylcyclohexane, dodecylcyclohexane, heptadecylcyclohexane, nonadecylcyclohexane, 1-eicosene, 1-docosene, 1-hexadecyne and 1-octadecyne (all from ChemSampCo, Dallas, TX) and 1-hexadecene, 1-octadecene, and 1-nonadecene (all from Fluka Chemical, Milwaukee, WI). All standards were used without additional purification, prepared in n-heptane (Sigma-Aldrich, St. Louis, MO).

Four different military fog oils were studied in this work. All samples were obtained from FO stores at different military installations. Two FOs produced after 1986 are defined as new and are referred to as NFO1 and NFO2 while two old FOs manufactured prior to 1986 are termed OFO1 and OFO2. All FO were analyzed as 1:10 dilutions in n-heptane.

The GCxGC system consists of an Agilent 6890N gas chromatograph retrofitted as a LECO comprehensive GCxGC-FID, which includes a dual-stage thermal modulator and a secondary oven for second column temperature control. The GC is equipped with a split / splitless injector and an Agilent 7683 autosampler. The benefit of this system is that the secondary oven and the modulator can be heated independently from the primary oven. This allows tuning the temperatures individually to achieve the optimal resolution.

Column specifications and separation parameters are listed above for the conventional column set and for the inverse column set. The conventional set consisted of a non-polar 100% dimethylpolysiloxane phase for volatility separations in the first dimension followed by a 50% diphenyl 50% dimethyl polysiloxane phase for polarity separations in the second dimension. Three different temperature programs were employed, a slow 1.5 ˚C/min ramp rate for maximum separations in the first dimension, a fast 5 ˚C/min to shorten the analysis time, and a polycyclic aromatic hydrocarbon (PAH) separation with a larger temperature difference between the primary and secondary ovens. In the inverse set, the first column was a 100% methylphenylpolysiloxane for polarity separations in the first dimension and the second non-polar column had a 100% dimethylpolysiloxane stationary phase and a slow 1.5 ˚C/min ramp rate was used. The modulation time was selected from 5 to 7 s to fill up the entire 2D retention plane with minimal wraparound effects.

In an attempt to pre-fractionate the FO to simplify GCxGC-FID analysis, a silica gel separation similar to those used by Reddy et al. (2007) and Glenn et al. (2003) was performed. Plastic pipettes with an inner diameter of 1/2 inch were filled with 1 g silica gel (100-200 mesh, Sigma-Aldrich) in the first separation step and 1 g silica gel coated with silver nitrate (~10 wt.%, Sigma-Aldrich) for the second separation step. The silica gels were pre-conditioned with 2 mL n-hexane (Spectrum, New Brunswick, NJ). After introduction of FO, the silica gel was eluted with 6 mL n-hexane to obtain Fraction F1 followed by elution with 6 mL of a 1:1 mixture of n-hexane:dichloromethane to obtain Fraction F2.. Both fractions were concentrated to 1 mL with a Zymark TurboVap II (Caliper Life Sciences, Hopkinton, MA). F1 was loaded onto the silver-impregnated silica gel column. Fraction F1.1 was obtained by elution with 4 mL n-hexane followed by elution with 6 mL dichloromethane/acetone (9:1 ratio) to obtain Fraction F1.2. These fractions were concentrated to 1 mL prior to analysis. The fractions were expected to contain saturated aliphatics in F1.1, unsaturated aliphatics and monoaromatics in F1.2, and aromatics and more polar compounds in F2.

4. RESULTS AND ACCOMPLISHMENTS

4.1 S&O Release and Deposition in the Field During Simulated Training Events

4.1.1 Green Smoke

The May experiment with green signaling smoke collected the dye settling from the sequential release of seven grenades, 1 m from the release point. Figure 9 shows the vials containing the extracted and concentrated solutions from collection media comparing the different types of ACF, aluminum foil, and GFF. All of these filters were cut to the same nominal size, 30.25 cm^2, as measured by a ruler along the edges.

Figure 9. Extracted samples from deposition of seven green signaling grenades. From left to right, the collection media were oxidized ACF, basic ACF, ACF-15, foil, GFF, and ACF-7.

Measurement of the green dye peak areas allows a direct comparison among these filters. The difference in the amount of green dye that is collected and extracted from these filters is striking. The oxidized ACF, basic ACF, and ACF-15 sample extracts contained no green dye, indicating that these filters either did not collect any green dye in the field or the dye could not be extracted using a dichloromethane soak. The green dye (Figure 10), also known as 1,4-bis[(4-methylphenyl) amino] anthracenedione or Solvent Green 3, may be strongly bound to the surface modified oxidized and basic ACF. It may also get trapped in the porous ACF-15 where it cannot be removed easily by a solvent rinse. Only a complex thermal desorption experiment would determine the actual amount of dye present, but the application of an electric field may degrade the dye before extraction. In contrast, the foil had a green dye peak area of 650,000, the GFF had a green peak area of 1,000,000, and the ACF-7 had a peak area of 2,500,000. The foil and GFF are inert substrates and will collect deposited chemicals without confounding issues of chemical interactions. While foil is a nonporous surface, GFF will have more surface area for trapping dye. The numbers illustrate that GFF can trap, hold, and release more dye than the foil, illustrating that porous substrates are better than foil. Under the same conditions, however, ACF-7 is the best performer. It can collect and release the largest amount of green dye. The activated carbon surface, together with the numerous pores for increasing the filter surface area, creates the best media for organic dye collection. It has a larger pore size than ACF-15, which can hold the chemical but will also allow the dichloromethane to easily remove the dye for analysis. The pores and the

sorptive surface serve to trap chemicals and prevent volatile or mechanical losses due to wind. Based on these numbers, ACF-7 represents the best substrate for green dye collection.

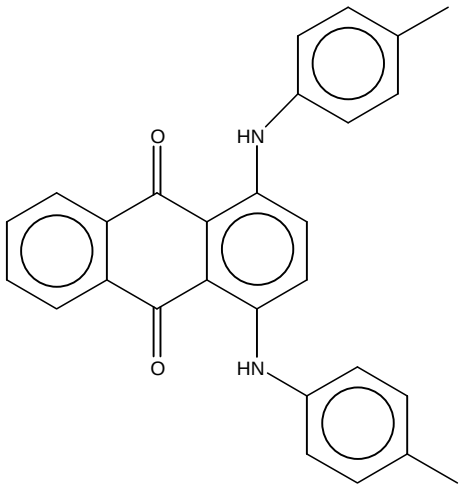

Figure 10. Green dye chemical structure.

Unfortunately, these ACFs are difficult to fabricate and acquire. Therefore, commercial filters that are readily available were tested. The August experiment with green signaling smoke collected the dye settling from the sequential release of 20 grenades, 25 m from the release point. Figure 11 shows the vials containing the extracted and concentrated solutions from collection media comparing the different types of filters. These vials are arranged in visual order, from clear to greenest. This is also the order in which they were analyzed by GC/MS.

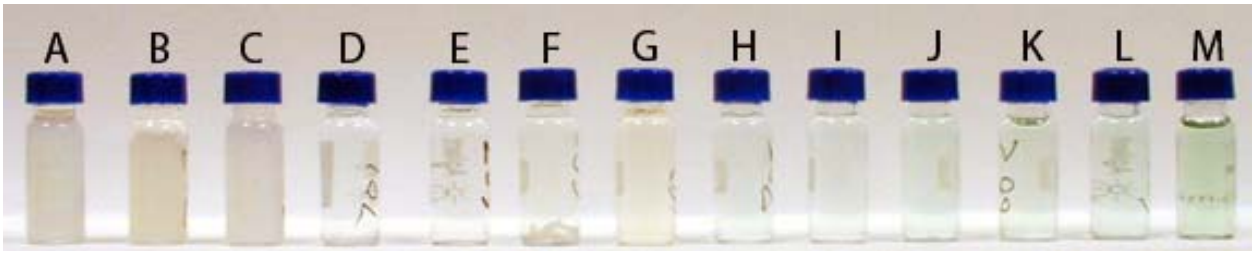

Figure 11. Extracted samples from deposition of 20 green signaling grenades onto different filters. A=Supor, B=ICE450, C=SB6407, D=Fiberfilm, E=FP450, F=Versapor, G=Tuffryn, H=Nylaflo, I=GFF, J=Metricel, K=foil, L=Emfab, M=PTFE.

Table 5 lists the green dye peak areas collected and extracted by these filters. The peak areas are divided by the filter nominal size. Only Metricel, Emfab, and PTFE show any collection of green dye at 25 m away from the release point. From Table 5, it is noted that these filters are hydrophobic substrates, Emfab and PTFE, due to the presence of the fluoroethylene. PTFE collects and releases the most green dye; more than ten times the amount observed in Emfab and Metricel. The dichloromethane extraction process completely degrades Supor, ICE450, and SB6407. These samples become viscous and cloudy, and are filled with the monomer from the filter substrate and support structure. Due to this contamination and from the lack of green color, these samples were not analyzed further.

Table 5. Green dye peak area / filter areas for all filter types.

Filter type	Peak area / filter area
Supor	degraded
ICE450	degraded
SB6407	degraded
Fiberfilm	0
FP450	0
Versapor	0
Tuffryn	0
Nylaflo	0
GFF	0
Metricel	3.6
Foil	0
Emfab	3.4
PTFE	36.3

4.1.2 Yellow Smoke

Initial May experiments with yellow signaling smoke collected the dye settling from the sequential release of 20 grenades, 5meters from the release point. Figure 12 shows a bar graph of the yellow dye peak areas. All of the solid substrates have nearly the same nominal size, 78 – 80 cm^2, but heptane in the jar has an available surface area of only 44.2 cm^2.

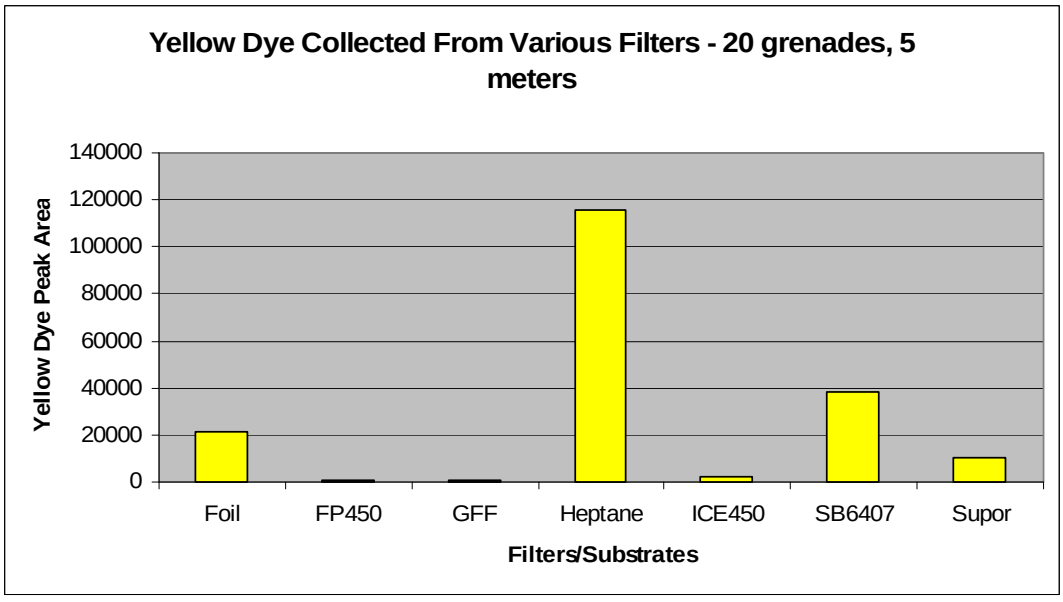

Figure 12. Yellow dye peak areas collected on different substrates using 20 grenades at 5 m from the release point.

Of the solid substrates, only SB6407, Supor, and aluminum foil collected an appreciable amount of yellow dye. After the green dye experiment described above, the SB6407 and Supor samples were filtered with 0.22 μm filters to remove the viscous component. This allowed injection of

these samples to occur. A jar of heptane, however, does the best job of collecting yellow dye (Figure 13), also known as 1H-indene-1,3(2H)-dione, 2-(2-quinolinyl) or Quinoline Yellow. Since heptane is a nonpolar liquid, it has essentially unlimited capacity to collect and store dye, compared with solid substrates, which are limited by size and surface area. It is recognized that the transport and application of heptane can limit the use of this solvent in the field.

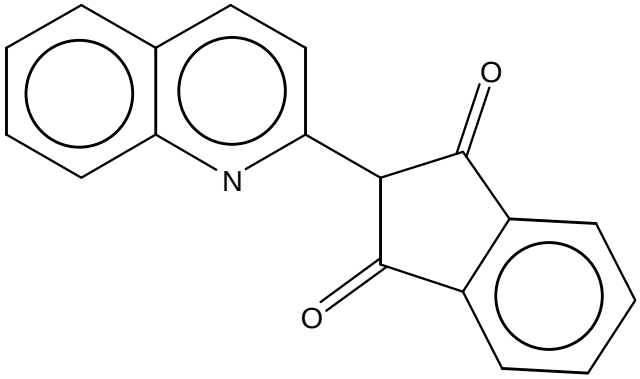

Figure 13. Yellow dye chemical structure.

A second experiment was performed using 20 yellow signaling grenades with collection substrates arranged 25 m from the release point. At 25 m from the release point, the yellow cloud is expected to be more dilute and the fraction of settleable components to be proportionately less. Figure 14 shows a bar graph of the yellow dye peak areas collected and extracted during this experiment.

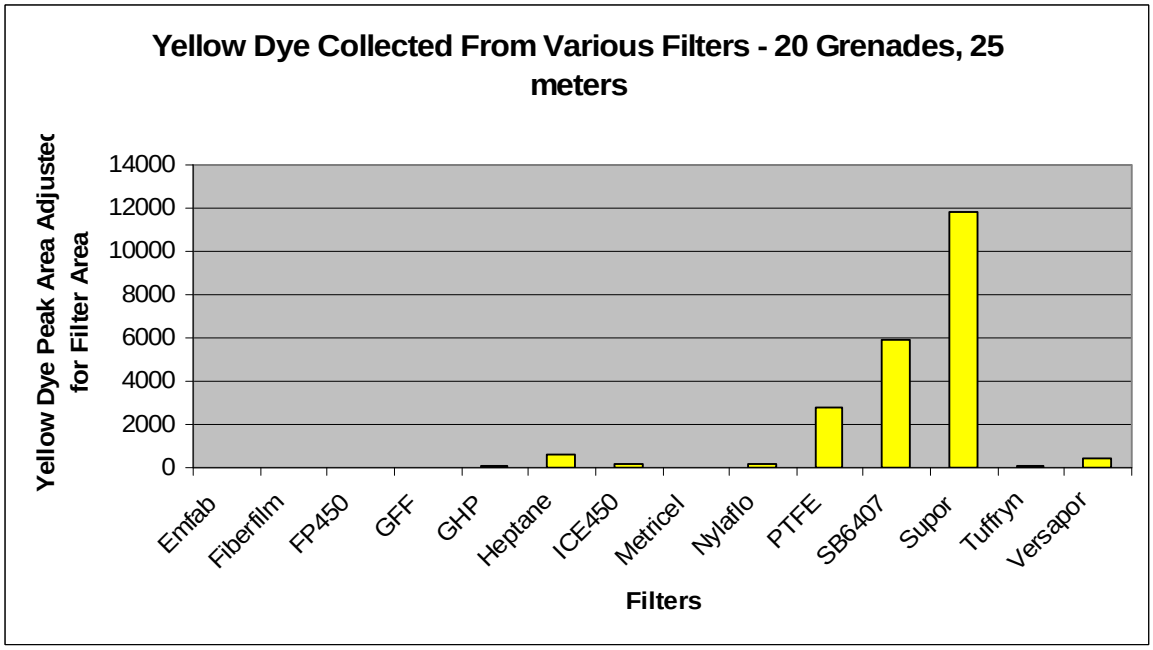

Figure 14.Yellow dye peak areas collected on different substrates using 20 grenades at 25 m from the release point.

The amount of yellow dye that settles at 25 m is approximately an order of magnitude less than that which deposits at 5 meters. Foil was not included in this series. Again, Supor and SB6407 were among the two best performers, and similar to the green dye data, PTFE can also collect yellow dye. Curiously, Supor collected nearly the exact amount at 25 m as it did at 5 m (a peak area of 11000). One possibility is that Supor is completely saturated at 5 m and collects yellow dye so well at 25 meters, that it is also saturated at this distance as well. In this instance, it is suggested to use a substrate with higher capacity at close distances and the superior collecting power of Supor at distances farther from the grenade release point. At 25 meters, it is also noted that heptane is no longer the best substrate. It collects far less than the solid substrates at this distance. This could be due to a physical process where the deposition of chemicals over a jar of heptane is disrupted by the heptane volatility, or it could simply denote the difficulties of the uncontrollable nature of field studies.

4.1.3 Red Smoke

The dye in red smoke in these older grenades is Disperse Red 9, or 1-methylamino anthraquinone, shown in Figure 15. Due to difficulties in obtaining a pure sample of this dye for standards and due to its lack of toxicity (shown below), this dye was not studied further for extraction from collection substrates.

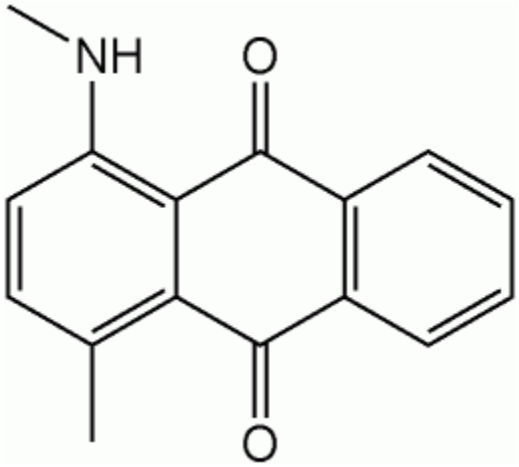

Figure 15. Red dye chemical structure.

4.1.4 Fog Oil – Field Release

The May FO experiment collected the hydrocarbon components settling at 5 m from the release point using either a 15- or 18-min fogging time. Figure 16 shows the FO collected and released by the ACFs, aluminum foil, GFF, and both water and heptane.

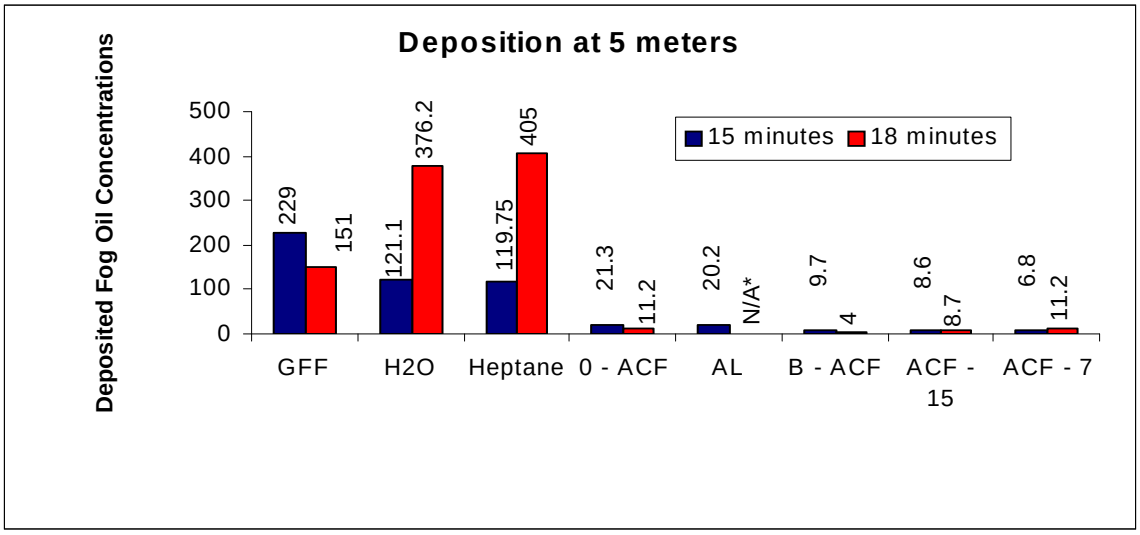

Figure 16. FO peak areas collected on different substrates using 15- and 18-min fogging times at 5 m from the release point.

Both water and heptane were examined in this experiment. Heptane does an excellent job at reproducing the exact amount of oil that deposits and collects on a water surface. The volatility of FO may limit glass fiber filter's effectiveness, while inert substrates such as aluminum foil coupons do not retain the FO deposition. The ACFs likely retain the FO too strongly and give poor recovery.

4.1.5 Fog Oil – Chamber Release

Field release of S&O is uncontrollable. Drift, diffusion, and deposition characteristics are likely to fluctuate under the vagaries of weather conditions. In an effort to better control the amount of S&O that each filter experiences and, further, to ensure that each filter experiences identical deposition conditions, a set of filters was submitted to FO released within the fogging chamber. An experiment was conducted using 2 min of fogging and a 9-hour settling time. A set of filters was placed on the floor of the chamber as shown in Figure 17 so that all were subjected to the same amount of fog.

Table 6 lists the amount of FO that was extracted from the filters. The concentration of FO was determined by GC/MS using a calibration curve made from known concentrations of FO in heptane. The filter results are listed from the smallest to the largest concentration in column two. Column three calculates the total amount of FO extracted from the filter. Column four is the amount of FO collected per filter area. In this experiment, and as seen in Figure 17, the size of some of the filters was increased to 8 in. x 10 in. sheets. Therefore, the values listed in column four show the most relevant parameter. If the sorbents are ranked from least to most FO collected, foil and Fiberfilm are the worst performers, while Metricel easily outperforms all others. GHP, SB6407, and heptane are the next best collectors of FO. Metricel and GHP are polypropylene and this polymer seems to work very well. Metricel is hydrophobic, however, as compared to the hydrophilic GHP, which may be the reason for the large difference in collecting the hydrophobic FO.

Figure 17. Filters placed on the floor of the chamber for a fogging event.

Table 6. FO extracted from filters from the fogging chamber.

Filter	Concentration [mg/ml]	Amount on filter [mg]	Amount/filter area [mg/cm2]
GFF small	0.2	0.4	0.0042
ICE 450	0.3	0.5	0.0064
SB 6407	0.3	0.5	0.029
FP 450	0.3	0.6	0.0076
Foil	0.4	0.8	0.0016
Fiber Film	0.7	1.4	0.0027
Heptane	0.8	1.6	0.036
Emfab	0.9	1.7	0.0033
PTFE	1.2	2.4	0.0048
Tuffryn	1.2	2.4	0.0048
GFF large	1.4	2.7	0.0052
Versapor	1.4	2.9	0.0056

Filter	Concentration [mg/ml]	Amount on filter [mg]	Amount/filter area [mg/cm2]
Nylaflo	2.4	4.8	0.0096
Supor	3.3	6.6	0.013
Metricel	7.3	14.5	0.18
GHP	7.3	14.6	0.028

4.1.6 Two-Dimensional Separations

FO is an exceedingly complex hydrocarbon mixture that may contain as many as one million components. Typical GC/MS analysis of FO results in an unresolved hump where individual components are unable to be extracted for identification. New techniques in separation science are enabling separations in two-dimensional (2D) space where the first dimension uses differences in volatility among compounds and the second dimension uses differences in polarity. Analysis of FO using 2D GC/FID provides a glimpse of the sheer sample complexity. Figure 18 shows a 2D GC/FID separation of HOC FO. Each peak represents, at best, a single component of FO. The axis that reaches from foreground to background is the volatility dimension. Lighter, more volatile compounds elute toward the foreground, while heavier denser compounds elute toward the background. The axis from the right to the left side of the figure is the polarity dimension. Nonpolar compounds appear toward the right side and more polar species elute toward the left side of the figure. The goal in this type of analysis is to spread the peaks out as much as possible in this 2D space to resolve the compounds from one another.

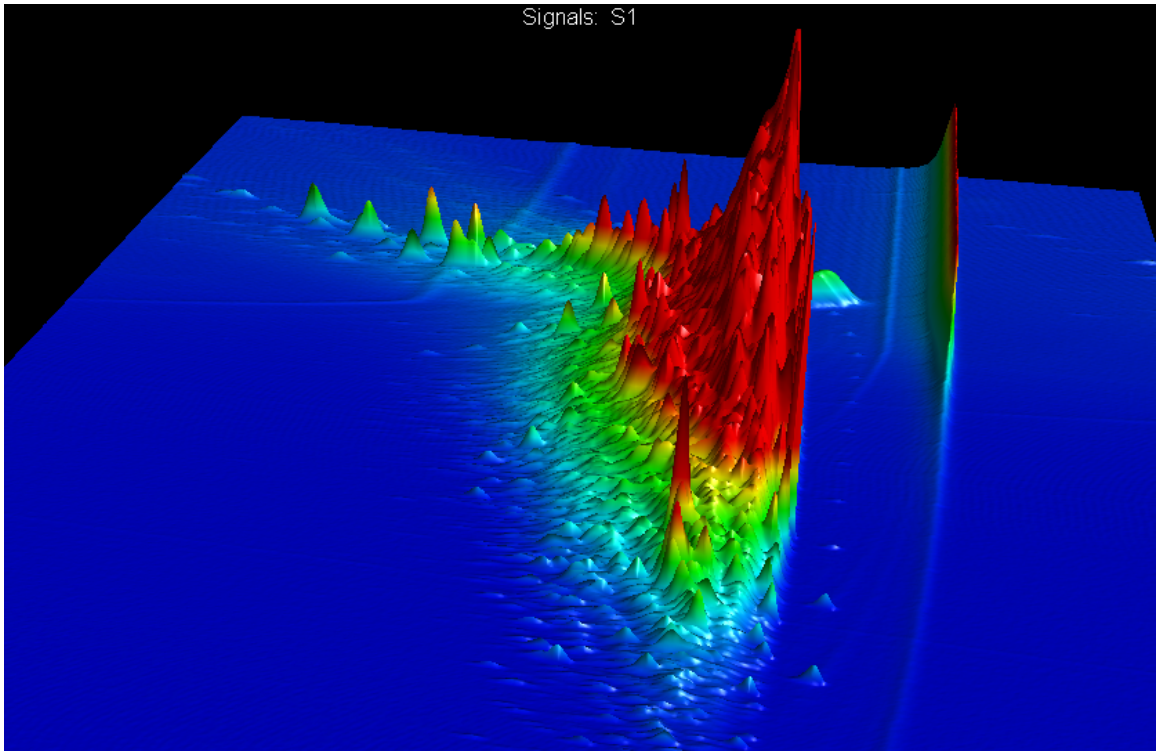

Figure 18. 2D GC/FID analysis of HOC FO.

Analysis of the sample extracts from the filters after chamber deposition of FO can be performed by 2D GC/FID. If the data are plotted as a surface plot, differences can be seen in the oil fractions that are collected on each filter. Figure 19 illustrates the surface plots of oil extracted from each filter sample. The x axis is volatility and the y axis is polarity. Dark blue is background, while lighter blue to green to yellow and finally to red indicates a progressive increase in the peak intensity.

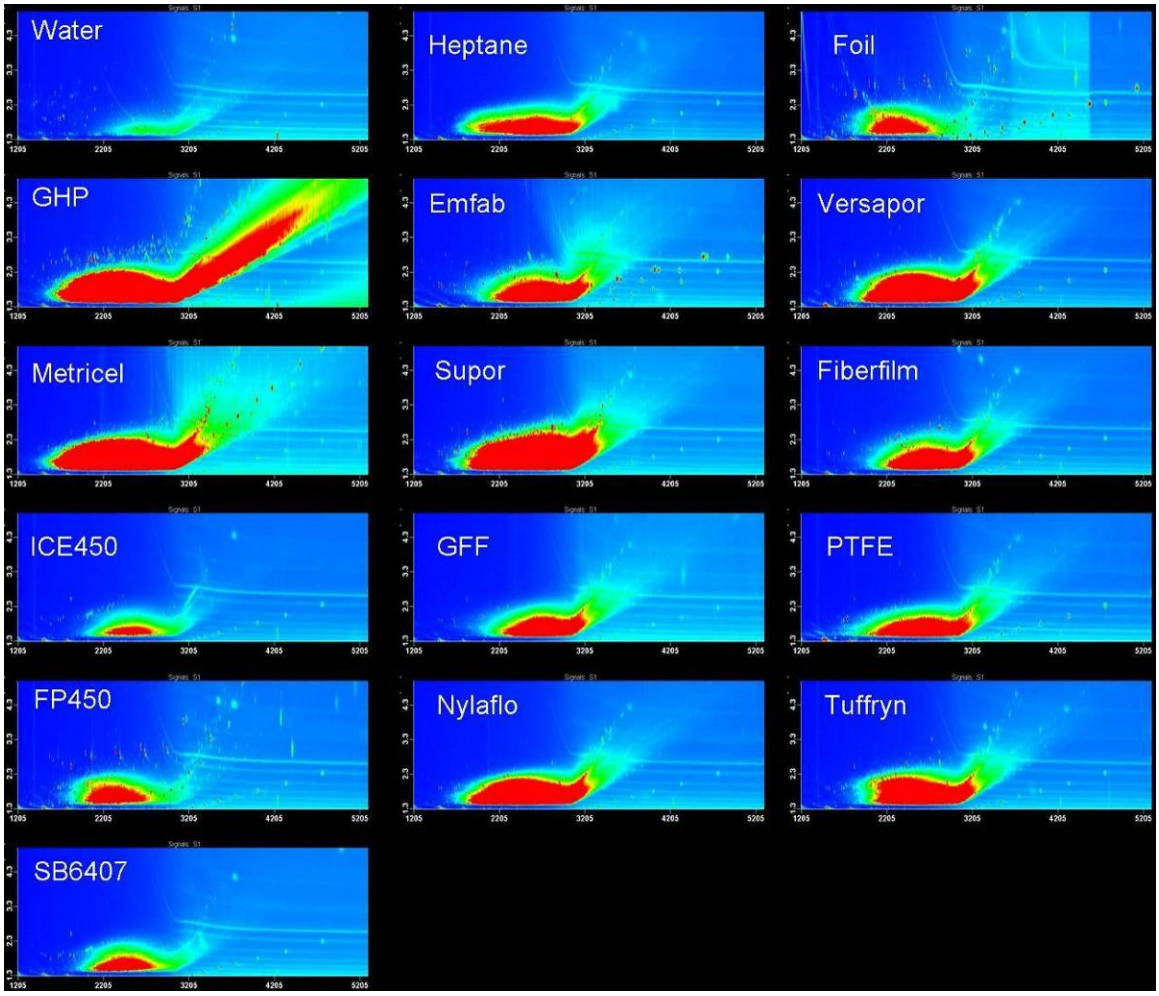

Figure 19. Surface plots of the 2D GC/FID analysis of oil extracted from fibers from chamber deposition.

Several characteristics can be noted when the data are displayed in this manner. The green area, signifying oil that deposits on water, elutes from 2205 to 3205 seconds on the volatility (x) axis and extends up to only 1.8 seconds on the polarity (y) axis at 2800 seconds. Most of the filters collected a far greater fraction of oil, including the more volatile fraction below 2205 seconds. As the most extreme examples, GHP and Metricel collected not only the most volatile species below 2205 seconds but can also collect more of the denser compounds that elute after 3205 seconds. So, while these two filters collected the most fog oil, they did not perform well at predicting the fraction that will deposit on water. To a lesser degree, Nylaflo and Tuffryn collected compounds that are more polar, as illustrated by an oil peak that extends to 2.3 seconds in the polarity dimension at 2800 seconds. While the oil peak in water is centered around 2700 seconds, foil

and FP450 are centered near 2400 seconds with a tendency toward collecting the lower boiling point compounds of oil. By visual comparison, the filters that best reproduce the water deposition peak are GFF, Fiberfilm, and Emfab, where GFF is the best substrate. Curiously, all three of these filter types are predominantly borosilicate glass fibers, which seem to have the ideal behavior for mimicking the deposition of hydrocarbons on water.

4.1.7 Deposition at Distance From Generator Vehicle

Average climatic conditions for the May and August field exposure trials are shown in Table 7. In both the May and August field exposure experiments, no fog oil was observed in control samples placed upwind of the generator (Table 7). In May, substantial amounts of NFO deposited onto water samples at the 5 m test position, but by 50 m, accumulation of NFO was considerably lower, and farther than 50 m from the generator, no oil was detected in sampling jars filled with water (Table 7). Deposition at the 5 m sampling location ranged from 0.23 mg NFO after 3 min of fogging to 7.4 mg NFO after 18 min of fogging. During the August trip, accumulations of NFO were less at all times and distances. At the 5 m distance, for instance, 3 min of fogging resulted in nearly a 10 fold decrease in NFO on the water surface relative to deposition observed in May. This trend was even more pronounced for the 18 min fogging test. Fogging for 30 min and 60 min resulted in deposition of 230 and 430 μg NFO, respectively, at 5 m (Table 7). Not surprisingly, no NFO was detected on the 50 m August samples and no NFO was detected at any greater distance. Deposition rates, calculated using a 44.2-cm^2 sampling jar surface area, were variable at the 5-m sampling location in May, but relatively constant over time in August, ranging from 1.4 to 2.4 mg NFO/m^2/min (Table 7). However, deposition rates at the 25-m sampling location in August ranged widely from 0.14 to 2.6 mg NFO/m^2/min.

Table 7. Mean (± S.E.) climatic conditions during May and August field trials.

	May	August
Wind speed (m/s)	4.74 ± 0.09	2.10 ± 0.09
Air temperature (°C)	18.8 ± 0.1	24.7 ± 0.1
Relative humidity (%)	50 ± 1	88 ± 1
Solar radiation (W/m^2)	542 ± 40	110 ± 16.7
Atmospheric pressure (atm)	1.002 ± 0	0.996 ± 0

Table 8. New fog oil (NFO) deposition, in mg oil extracted from the entire sample jar, at different fogging times and distances from the generator release point during field experiments in May and August 2003. Values shown in parentheses are deposition rates (mg NFO/m^2/min).

May			August				
D(m)	3 min	18 min	D(m)	3 min	18 min	30 min	60 min
-50	nd	nd	-50	nd	nd	nd	nd
5	0.23 (17)	7.4 (94)	5	0.018 (1.4)	0.19 (2.4)	0.23 (1.8)	0.43 (1.6)
50	nd	0.11 (1.4)	25	0.034 (2.6)	0.025 (0.31)	0.019 (0.14)	sl
100	nd	nd	50	nd	nd	nd	nm
500	nd	nd	100	nd	nd	nd	nm
800	nd	nd	250	nm	nm	nm	nm

Notes: nd = <0.1 mg for May, <0.01 mg for Aug; nm = not measured; sl = sample lost; D(m) = distance in m downwind from the generator

In field exposure experiments, deposition of fog oil increased both with increasing generation time and with decreasing distance from the generator, as was expected. Oil was detectable in water samples up to 50 m downwind of the generator (May experiment). In addition, deposition rates were highly variable, as has been seen in other studies (Douglas et al., 2006), both for the same distance in different tests (May versus August, 5 m) and over time at constant distance (5 m in May). The May and August deposition rates for the 18 min exposures were quite different, with May having an approximately 40 fold higher deposition rate. This may, in part, be explained by differences in ambient conditions, for example May had higher wind and solar radiation, and lower temperature and relative humidity than did August. In addition, based on visual observations during fog production, it is possible that physical ejection of large oil droplets from the generator nozzle caused the drastic difference in some of the oil deposition rates at this close range. These findings, along with our field observations illustrate the difficulties inherent in quantifying FO deposition in open areas subject to variable winds. Oils are perhaps the most complex and variable mixtures to evaluate toxicologically for several reasons, including the large number of chemical constituents, the varying array of physicochemical properties of each individual component, inconsistent and non-reproducible matrix preparation, and even batch irregularities in bulk oil composition (Singer et al. 2000). Military FO composition can vary from manufacturer to manufacturer and from lot to lot (Langford 2004). This study has shown that fog oil can be measured in water as far as 50 m downwind of a generator. The FO deposition collection methods in this research were representative of the passive exposures organisms may experience in the field. Attention was paid to collect only the deposition fraction rather than an active air sampling system that would collect all oil components from the atmosphere. Previous field deposition and biological effects studies have used active means of collecting fog oil over distance and time (i.e., air sampling with dust sensors or air drawn through filters to infer effects on vegetation), thus making correlations of effect with quantification of exposure difficult (Schaeffer et al. 1986).

4.2 Field Toxicity on Aquatic Organisms During Simulated Training Events

4.2.1 D. magna mortality with fog oil

For both May (Table 9) and August (Table 10) exposures, control (-50 m) survival was 100% and no toxicity or floaters were observed, indicating that the methods for water temperature control in the field were sufficient and samples were well positioned to reflect any environmental effects in the absence of FO. Five m downwind, *Daphnia magna* mortality was significantly increased relative to controls ($p<0.05$) at 48 h following all but one (August, 3 min) exposure duration in both May and August experiments. The highest mortality observed for all experiments was 85% in the May, 5 m, 3 min station, where only 0.23 mg NFO was deposited. Although several sampling locations had nominal increases in mortality, no statistically significant increases in mortality were observed in either month beyond the 5 m sampling location. No mortality or floaters were observed 24 or 48 h following exposures 500 or 800 m downwind during the May experiment, so these sampling locations were excluded for the August exposures.

No organisms stuck in surface film (floaters) were observed in any of the upwind control replicates (-50 m, Tables 8 and 9); however, floaters were observed at several downwind stations. This sub-lethal effect significantly increased at 5 m for all fogging durations, with the exception

of the May 3 and 18-min exposures, where after 48 h, only 20 and 5% of the organisms were stuck in the surface oil film, respectively. Curiously, in nearly all cases, the percentage of floaters at 24 h was greater than or equal to that observed at 48 h post-exposure. Significant increases in floater percentages were observed as far as 50 m downwind of the generator. This would indicate that *D. magna* are sensitive to the presence of surface oils even at non-detectable levels of FO (3 min, 50 m, May).

Table 9. Fog oil (FO) deposition and associated *D. magna* mortality and floaters (n=20) at 24 and 48 h post-exposure in May.

	3-min FO deposition				
Distance	**FO deposited**	**24-hour**		**48-hour**	
(m)	**µg/L**	**%mortality**	**%floaters**	**%mortality**	**%floaters**
-50	nd	0	0	0	0
5	0.76	30*	80*	85*	20
50	nd	0	35*	0	0
100	nd	0	15	0	0
500	nd	0	0	0	0
800	nd	0	0	0	0
	18-min FO deposition				
Distance	**FO deposited**	**24-hour**		**48-hour**	
(m)	**µg/L**	**%mortality**	**%floaters**	**%mortality**	**%floaters**
-50	nd	0	0	0	0
5	24.6	15	60*	50*	5
50	0.36	0	85*	10	50*
100	nd	0	25	10	10
500	nd	0	0	0	0
800	nd	0	0	0	0

Table 10. Fog oil (FO) deposition and associated *D. magna* mortality and floaters (n=20) at 24 and 48 h post-exposure in August. nd = not detected, * indicates value is significantly different from control (-50 m).

	3-min FO deposition				
Distance	**FO deposited**	**24-hour**		**48-hour**	
(m)	**µg/L**	**%mortality**	**%floaters**	**%mortality**	**%floaters**
-50	nd	0	0	0	0
5	60	15	75*	20	75*
25	113	10	0	10	0
50	nd	5	0	10	0
100	nd	15	0	20	0
250	nd	0	0	0	0
	18-min FO deposition				
Distance	**FO deposited**	**24-hour**		**48-hour**	
(m)	**µg/L**	**%mortality**	**%floaters**	**%mortality**	**%floaters**
-50	nd	0	0	0	0
5	633	5	80*	55*	35*
25	283	5	0	5	5
50	nd	10	10	15	0
100	nd	0	0	5	0
250	nd	0	0	0	0

	30-min FO deposition				
Distance	**FO deposited**	**24-hour**		**48-hour**	
(m)	**µg/L**	**%mortality**	**%floaters**	**%mortality**	**%floaters**
-50	nd	0	0	0	0
5	766	5	75*	65*	25*
25	63	15	0	15	0
50	nd	0	0	0	0
100	nd	5	0	15	0
250	nd	0	0	0	0
	60-min FO deposition				
Distance	**FO deposited**	**24-hour**		**48-hour**	
(m)	**µg/L**	**%mortality**	**%floaters**	**%mortality**	**%floaters**
-50	nd	0	0	0	0
5	1433	15	75*	65*	30*

Notes: nd = not detected, * indicates value is significantly different from control (-50 m).

In field exposure experiments, deposition of fog oil increased both with increasing generation time and with decreasing distance from the generator, as was expected. Oil was detectable in water samples up to 50 m downwind of the generator (May experiment). In addition, deposition rates were highly variable, as has been seen in other studies (Douglas et al. 2006), both for the same distance in different tests (May versus August, 5 m) and over time at constant distance (5 m in May). The May and August deposition rates for the 18 min exposures were quite different, with May having an approximately 40-fold higher deposition rate. This may, in part, be explained by differences in ambient conditions; for example, May had higher wind and solar radiation, and lower temperature and relative humidity than did August (see Table 7. In addition, based on visual observations during fog production, it is possible that physical ejection of large oil droplets from the generator nozzle caused the drastic difference in some of the oil deposition rates at this close range. These findings, along with our field observations illustrate the difficulties inherent in quantifying FO deposition in open areas subject to variable winds.

In field exposures, we observed increased mortality of *Daphnia magna*, relative to upwind controls, albeit only at 5 m downwind. In addition, the number of floaters increased in relation to controls as far as 50 m downwind of the generator. While mortality was quite low beyond the 5 m sampling location, the floater endpoint was more sensitive. The fact that floaters occurred in our test chambers as far as 100 m downwind from the generation point (May) suggests the possibility that the sub-lethal floating behavior by *D. magna* is even more sensitive to the presence of surface oils than the chromatographic analysis method used. In experiments by Poston et al. (1986), daphnids were caught in the surface film at concentrations as low as 30 µg total fog oil/L, in agreement with our field experiments. Poston et al. (1986) suggested that this effect was due to the ingestion of oil microdroplets, which accumulate in the individual and ultimately induce buoyancy.

4.2.2 D. magna Mortality With Fog Oil Plus Graphite

While *D. magna* experienced significant mortality at a distance of 5 m from the release of the fog oil obscurant for the 3 and 18-min durations in May (Table 9), no significant mortality was observed in the 48 h following a 13.5-min fog oil plus graphite exposure in May. However, August

experiments yielded significant mortality of *D. magna* at 5 m for the 18- and 60-min fog oil plus graphite exposures (Table 11).

Table 11. % *D. magna* mortality for (n=20) exposed to fog oil plus graphite in August 2003 (* indicates significant difference from the control, ns = no sample, [a]n=15).

	3 min		18 min		30 min		60 min	
dist.	24	48	24	48	24	48	24	48
-50	0	0	0	7[a]	ns	ns	0	0
5	0	5	5	35*	ns	ns	30 [a] *	55 [a] *
25	0	0	5	25[a]	ns	ns	0	10
50	5	5	10	0	ns	ns	0	0
100	0	0	0	0	ns	ns	0	5
250	0	0	0	5	ns	ns	0	10

In the August 18 min fog oil plus graphite exposure, 90% (24 hours) then 5% (48 hours) of *D. magna* were caught. Forty % (24 hours) and 13% (48 hours) of *D. magna* were observed caught following the 60 min fog oil plus graphite exposure in August (Table 12). For all obscurant exposures, *D. magna* was never stuck at the surface of any control replicates.

Table 12. % *D. magna* (n=20 unless indicated) caught in surface film 24-and 48-hours following exposure to fog oil plus graphite in August 2003 (*indicates significantly different from the control (p=.05), [a]n=15).

	3 min		18 min		30 min		60 min	
dist.	24	48	24	48	24	48	24	48
-50	0	0	0	0	ns	ns	0	0
5	25	0	90*	5	ns	ns	40*	13
25	0	0	0	0	ns	ns	20	0
50	0	0	0	0	ns	ns	0	0
100	0	0	0	0	ns	ns	20	0
250	0	0	0	0	ns	ns	0	0

4.2.3 D. magna Fecundity With Fog Oil and Fog Oil Plus Graphite

Daphnia magna fecundity was tested following 18-min fog oil and 18-min fog oil plus graphite exposures in August. No significant reductions were observed in adult mortality, number of neonates per surviving adult, or total number of neonates produced in fog oil experiments (Table 13a). However, fog oil plus graphite yielded significant adult mortality (25% of adults) at 5 m (48 h). Still no significant difference was seen in total neonate production or number of neonates per surviving adult (Table 13b). Significant numbers of adults and neonates were found caught in the surface film at 5 m in both experiments. Seventy-five percent of adults and 100% of neonates at both 24 and 48 h were observed caught following the 18-min fog oil exposure (Table 14a). Ninety percent of adults and 100% of neonates were caught 24 h following the 18-min fog oil plus graphite exposure. Despite the substantial difference in the mean % adults and neonates caught at 48 hours, we did not find a statistical difference (Table 14b). This is likely a result of low statistical power due to the fact that only two control replicates produced neonates.

Table 13. *D. magna* fecundity 24 and 48 h following an 18-min exposure to (a) fog oil and (b) fog oil plus graphite (*indicates significant mortality compared to the control (p=.05), [a]n_{adults}=15, dist.= distance from release point in meters).

a: fog oil

	24 hrs		**48 hrs**		
	live		**live**		**total**
dist.	adults	neonates	adults	neonates	neonates
-50	15[a]	4	13[a]	11	15[a]
5	20	13	19	11	24
25	20	12	20	10	22

b: fog oil plus graphite

	24 hrs		**48 hrs**		
	live		**live**		**total**
dist.	adults	neonates	adults	neonates	neonates
-50	20	23	20	3	26
5	20	14	15*	16	30

Table 14. % *D. magna* adults and neonates caught in surface film 24 and 48 h following an 18-min exposure to (a) fog oil and (b) fog oil plus graphite (*indicates significant differences compared to the control (p=.05), [a]n_{adults}=15, dist.= distance from release point in meters).

a: Fog oil						**b: Fog oil plus graphite**				
	adults		**neonates**				**adults**		**neonates**	
dist.	24	48	24	48		dist.	24	48	24	48
-50	0[a]	0[a]	0	0		-50	0	0	0	0 (0/3)
5	75*	75*	100*	100*		5	90*	10	100*	50 (8/16)
25	0	0	0	0						

May and August field trials demonstrated that deposition of fog oil on aquatic surfaces produces both lethal and sublethal effects on *D. magna* in field exposures. Significant mortality was observed only relatively close to the release point. The floater phenomenon was observed to a lesser extent in the fog oil plus graphite exposures.

4.2.4 D. magna Mortality With Colored Smokes

Significant ($p<0.05$) mortality was observed for *D. magna* replicates at 1 m green (6 grenades) and yellow (16 grenades) smoke exposures. All *D. magna* (n=20) died within 24 h. (Animals were not exposed to red smoke at 1 m).

4.2.5 Colored Smoke Tests With Other Organisms

For *C. tentans*, *P. promelas, E. fonticola*, *N. topeka*, *O. mykiss*, and *R. pipiens,* mortality was recorded for each replicate at a minimum of 24 and 48 h. No significant mortality was observed for any species and exposure combination. For some fish and frog exposures, however, mortality was recorded at 72 and 96 h. All red, green, and yellow colored smoke exposures resulted in no

significant mortality in *C. tentans*, *P. promelas, E. fonticola*, *N. topeka*, *O. mykiss*, and *R. pipiens,* at any distance as compared with the control group (see Appendix A, Table A1).

4.2.5.1 *Stuckenia pectinatus*

Except for the one yellow grenade exposure at 25 m, there were no significant differences in final shoot length of any of the exposed plants in relation to the controls (Table 15). All of the plants appeared to be normal after 48 h exposure, and all of the plants produced new lateral branches and underground rhizomes after 3 weeks of outdoor growth. No morphological deformities were noted in any of the leaf, stem, or root structures. Because almost all plants tended to put their growth resources into new biomass for lateral branching and rhizomes rather than into new length (initial lengths were 10-15 cm), the plants for the August exposures were analyzed for biomass rather than shoot length (Table 16).

Table 15. Total length (cm) of *S. pectinatus* after 48 h exposure to red, green, and yellow smoke grenades followed by a 3 week grow-out period (May). Initial stem lengths = 10-15 cm. Four plants were exposed at each distance. * indicates treatment mean is significantly different from the control.

Dist. (m)	1 Red Grenade	6 Red Grenades	1 Green Grenade	6 Green Grenades	1 Yellow Grenade	6 Yellow Grenades
-50	28.8	25.0	25.6	27.1	26.1	24.7
5	27.0	32.2	21.1	27.2	22.1	22.3
25	25.4	31.9	24.6	28.7	20.6*	26.5
50	26.2	30.5	26.3	25.4	22.4	24.5
100	26.1	26.7	21.1	26.5	24.6	21.6
250	25.1	26.5	28.0	25.2	24.0	23.5

After the grow-out period, all *S. pectinatus* plants appeared to be normal in terms of leaf, stem, and root structures and rhizome production. However, analysis of the data showed that the green and yellow grenades may have had a stimulatory effect on growth in relation to the controls (Table 16). The trend was similar, but not statistically significant, for the red grenades. A positive growth response to colored smokes may cause or aggravate nuisance aquatic plant problems in exposed waters.

Table 16. Biomass (g dry weight per plant) of *S. pectinatus* plants after 48 h exposure to red, green, and yellow smoke grenades followed by a 3 week grow-out period (Aug). Initial biomass = 0.16 g. Four plants were exposed at each distance. * indicates treatment mean is significantly different from control.

Dist (m)	20 Red Grenades	20 Green Grenades	20 Yellow Grenades
-50	1.26	1.17	1.44
5	2.20	2.98*	2.60*
25	2.43	2.64*	2.77*

Except for the 6 red grenade exposure at 250 m, there were no significant differences in chlorophyll content of any of the exposed *S. pectinatus* plants in relation to the controls (Table 17). The single significant variation from control was an increase in chlorophyll content, which suggests that in the random pick of plants for the jars at this distance, we happened to pick four plants that

were slightly greener than normal. At multiple and high number red, green, and yellow smoke grenade exposure levels there were no significant differences in chlorophyll content of any of the exposed plants in relation to the controls (Table 18).

Table 17. Chlorophyll content (mg chl. a/g fr. wt.) of *S. pectinatus* leaves after 48 h exposure to red, green, and yellow smoke grenades (May). Four plants were exposed at each distance. * indicates treatment mean is significantly different from control.

Dist. (m)	1 Red Grenade	6 Red Grenades	1 Green Grenade	6 Green Grenades	1 Yellow Grenade	6 Yellow Grenades
-50	0.97	0.70	0.86	0.89	0.79	0.76
5	0.80	0.90	0.77	0.87	0.69	0.76
25	0.94	0.90	0.74	1.00	0.64	0.93
50	0.82	0.74	0.75	0.98	0.85	0.62
100	0.88	0.82	0.69	0.89	0.85	0.66
250	0.87	1.03*	0.77	0.93	0.63	0.58

Table 18. Chlorophyll content (mg chl. a/g fr. wt.) of *S. pectinatus* leaves after 48 h exposure to red, green, and yellow smoke grenades (Aug). Four plants were exposed at each distance. * indicates treatment mean is significantly different from control.

Dist. (m)	20 Red Grenades	20 Green Grenades	20 Yellow Grenades
-50	1.4	1.0	0.8
5	1.2	1.0	0.6
25	1.2	1.1	0.6

4.2.5.2 *Pseudokirchneriella subcapitata*

There were no significant differences in chlorophyll content of any of the exposed jars with *Pseudokirchneriella subcapitata* in relation to the controls (Table 19).

Table 19. Chlorophyll content (µg chl. a/L) of *P. subcapitata* after 48 h exposure to red, green, and yellow smoke grenades (Aug). Four jars with *P. subcapitata* were exposed at each distance. * indicates treatment mean is significantly different from control.

Dist. (m)	20 Red Grenades	20 Green Grenades	20 Yellow Grenades
-50	186.6	163.3	198.7
5	175.0	131.4	159.8
25	163.6	181.5	159.8

4.2.6 Fog Oil Tests with Other Organisms

For *C. tentans* exposure, mortality was not significantly different from the controls in 48-hour acute field toxicity testing. For *P. promelas, E. fonticola*, *N. topeka*, *O. mykiss*, and *R. pipiens* exposures, mortality was not significantly different from controls in 48, 72, or 96-hour acute field toxicity testing. (see Appendix A, Table A2).

4.2.6.1 *Stuckenia pectinatus*

There were no significant differences in final shoot length of any of the exposed plants in relation to the controls (Table 20). All of the plants appeared to be normal after 48 h exposure to the fog oil, and all of the plants produced new lateral branches and underground rhizomes after 3 weeks of outdoor growth. No morphological deformities were noted in any of the leaf, stem, or root structures.

Because the plants tended to put their growth resources into new biomass for lateral branching and rhizomes in late summer rather than into new length (initial lengths were 10 – 15 cm), the plants for the August exposures were analyzed for biomass rather than shoot length. There were no significant differences in biomass between exposed plants and the controls (Table 21) even after an extended 120 min fogging time. After grow-out, all plants appeared to be normal in terms of leaf, stem, and root structures and rhizome production.

For the May experiments, except for the 18 min exposure at 5 m, there were no significant differences in chlorophyll content of any of the exposed plants in relation to the controls (Table 22). The significant value (0.52 mg chl a/g FW) is within the range of chlorophyll values for many of the other plants exposed to FO obscurant. For the August experiments, the 18 min (25, 50, and 500 m) and 30 min fog oil (50, 100, 250, 500 m) exposures showed significant differences from controls (Table 23). In some cases, the exposed plants had lower chlorophyll values than the controls; in others, the exposed plants had higher chlorophyll values than the controls.

Table 20. Total length (cm) of *S. pectinatus* stems after 48 h exposure to fog oil obscurant followed by a 3 week grow-out period (May). Initial stem lengths = 10-15 cm. Four plants were exposed at each distance. -50 m (50 m upwind) served as the control position.

Dist. (m)	Fog Oil 3 min	Fog Oil 18min
-50	26.2	24.9
5	25.6	23.5
25	21.2	23.2
50	23.0	23.1
100	25.3	24.4
250	27.7	24.2

Table 21. Biomass (g DW per plant) of *S. pectinatus* plants after 48 h exposure to fog oil obscurant followed by a 3 week grow-out period (Aug). Four plants were exposed at each distance. -50 m (50 m upwind) served as the control position.

Dist. (m)	Fog Oil 18 min	Fog Oil 30 min	Fog Oil 60 min	Fog Oil 120 min
-50	1.48	1.34	1.66	1.17
5	1.82	1.72	1.41	1.48
25	1.56	1.05	1.62	1.61
50	1.34	1.54	1.52	1.32
100	1.37	1.78	1.65	1.11
250	1.66	1.45	1.48	0.98
500	1.60	1.40	1.71	1.42
800	No sample	1.11	1.18	0.80

Table 22. Chlorophyll content (mg chl a/g FW) of *S. pectinatus* leaves after 48 h exposure to fog oil obscurant (May). Four plants were exposed at each distance. -50 m (50 m upwind) served as the control position. * indicates treatment mean is significantly different from control.

Dist. (m)	Fog Oil 3 min	Fog Oil 18 min
-50	0.52	0.76
5	0.56	0.52*
25	0.62	0.76
50	0.63	0.65
100	0.56	0.58
250	0.56	0.54

Table 23. Chlorophyll content (mg chl. a/g FW) of *S. pectinatus* leaves after 48 h exposure to fog oil obscurant (Aug.). Four plants were exposed at each distance. -50 m (50 m upwind) served as the control position. * indicates treatment mean is significantly different from control.

Dist. (m)	Fog Oil 18 min	Fog Oil 30 min	Fog Oil 60 min	Fog Oil 120 min
-50	1.2	1.5	1.1	1.3
5	1.3	1.3	1.2	1.3
25	1.5*	1.2	1.3	1.4
50	1.4*	0.5*	1.1	1.2
100	1.3	1.0*	1.2	1.2
250	1.3	0.6*	1.4	1.4
500	1.4*	0.6*	1.2	1.2
800	No sample	1.4	1.2	1.3

In these exposures, we observed no effects on growth in *S. pectinatus* attributable to FO obscurant exposure. The observed differences in *S. pectinatus* chlorophyll a content at the 5 m, 18 min exposure in May, the 25 m, 50 m, and 500 m 18 min exposures in August, and the 50 m, 100 m, 250 m, and 500 m 30 min exposures in August do not show any consistent pattern. Some differences are greater than those of the controls while others are less. In instances where the chlorophyll content is less (50, 100, 250, and 500 m at 30 min), there was no detectable FO deposition and, in one case where chlorophyll content increased (25 m, 18 min Aug), a significant amount

of FO was present at the water surface. For the 30 min exposures, even though there was statistical significance, no overt bleaching of the sago pondweed leaves was visible to the naked eye. In addition, no significant differences were noted at any of the exposures at 60 and 120 min, times in which prolonged exposure might be expected to produce bleaching from a combination of sunlight and the obscurant. If an enhancement in chlorophyll did occur because of exposure to the FO obscurant, this could possibly be construed as a positive effect. In another study, total chlorophyll content of *S. pectinatus* has been reported as averaging 0.81 mg/g FW (Madsen 1986). This value is intermediate between our May and August values, suggesting seasonal differences. Other studies have reported apparent temperature and irradiance, corresponding to seasonality, induced differences in *S. pectinatus* Ch a values (mean levels of 243 - 1005 µg/g FW) (Spencer and Anderson 1987; Spencer and Ksander 1987). We believe that observed differences in chlorophyll content can be attributed to sample variation and had no impact on overall growth of the plants.

4.2.6.2 *Pseudokirchneriella subcapitata*

Chlorophyll production was determined for *P. subcapitata* during August exposures. The only exposures to show a significant difference from the controls were the 60 min (50 and 250 m) and the 18 min (50 m) exposures (Table 24). The sites which most likely received the highest doses of the obscurants (5 and 25 m) showed no statistical differences from the control values, although there did seem to be a suppression of chlorophyll with 120 min FO obscurant exposure at 50 m. No apparent loss of chlorophyll was visible to the naked eye. In general, it did not appear that the obscurants had a significant negative effect on *P. subcapitata.*

Table 24. Chlorophyll content (µg chl a/L) of *P. subcapitata* after 48 h exposure to fog oil obscurant (Aug.). Four jars with *P. subcapitata* were exposed at each distance. -50 m (50 m upwind) served as the control position. * indicates treatment mean is significantly different from control.

Dist. (m)	Fog Oil 18 min	Fog Oil 30 min	Fog Oil 60 min	Fog Oil 120 min
-50	166.7	160.2	161.5	156.7
5	168.2	151.3	170.6	160.0
25	156.1	164.9	153.6	137.1
50	142.7*	157.2	135.4*	96.2
100	164.1	167.9	159.4	149.0
250	156.8	157.2	134.6*	121.9
500	170.3	156.4	152.5	140.3
800	No sample	151.3	148.2	161.2

In another study, low concentrations of BP light diesel (0.05%) and the oil dispersant BP1100X (0.005%), either alone or in mixture, stimulated the growth rate, biomass yield, chlorophyll a level and photosynthesis of the estuarine green alga *Chlorella salina*, while the same concentrations slightly inhibited algal respiration (Chan and Chiu 1985). Thus, low levels of hydrocarbons can induce either beneficial or deleterious sub-lethal effects. In these experiments, however, since FO was undetectable beyond 50 m, the differences in chlorophyll content observed is most likely attributed to sample variation.

It is known that FO from different manufacturers and lots have differences in chemical composition that confounds toxicity studies, as well as preventing the attribution of toxic effects to any single compound or class of compounds (Singer et al. 2000). These differences have been related to differing toxicity to *Daphnia magna* (Cropek et al. 2008), albeit primarily through physical contact. Both *S. pectinatus* and *P. subcapitata* inhabit the water column. Although S. *pectinatus* leaves, branches, and inflorescences can reach the surface, they usually do not. Because of inherent insolubility of FO obscurant and water, FO does not enter the water column, thus, *S. pectinatus* and *P. subcapitata* contact with FO obscurant is limited. Studies of toxicity of oils and water can involve effects of water soluble fractions (WSF) and water accommodated fractions (WAF) (Singer et al. 2000), that can influence bioavailability. Investigation of the amount of generated FO that dissolves into the water column (WSF) shows very little increase in the total organic carbon content of the water (Cropek et al. 2008), suggesting that the same may be true for FO obscurant and *S. pectinatus* and *P. subcapitata.*

Under the conditions employed here, there is no appreciable route of exposure of generated FO to *S. pectinatus* and *P. subcapitata* in field conditions. FO obscurant deposition decreases with distance from the source and aquatic ecosystems and FO obscurant interaction beyond 50 m appears minimal. Both plant species utilize carbon dioxide from the water column with *S. pectinatus* obtaining nutrients from sediments (Spencer 1990; Barko et al. 1991; Cronk and Fennessy 2001). *S. pectinatus* biomass production as observed in this study indicates that FO obscurant exposure did not interfere with nutrient uptake and subsequent growth. Further, the chlorophyll a values obtained for both species suggest that FO obscurant exposure at the relevant levels observed in military training events did not interfere with photosynthesis processes or cause tissue damage. Other environmental scenarios exist that could increase the bioavailability of deposited FO to underwater plants such as extreme water agitation that maximizes WAF and biodegradation or photolytic processes that transform hydrocarbons into water soluble chemical species. Continued research in these transport mechanisms would be valuable for a complete risk assessment of the environmental use of FO.

4.2.7 Fog Oil plus Graphite Tests With Other Organisms

For *C. tentans*, *P. promelas, E. fonticola*, *N. topeka*, *O. mykiss*, and *R. pipiens* exposures, mortality was not significantly different from the control specimens in 48, 72, or 96-hour acute field toxicity testing (see Appendix A, Table A3).

4.2.7.1 *Stuckenia pectinatus*

After the grow-out period, all plants appeared to be normal in terms of leaf, stem, and root structures and rhizome production. Analysis of the data shows that fog oil + graphite at an exposure time of 18 min may have reduced growth in relation to the controls at 5, 100, 250, 500, and 800 m (Table 25). However, the control value of 2.49 g dry weight per plant at that exposure time was the highest value of any of the control values for either the fog oil, fog oil + graphite, or even the red, yellow, and green grenades. It seems likely that the mean growth of the 18 min fog oil + graphite control plants is unusual and is probably not a realistic value upon which to compare the effects at the other distances. None of the other time or distance exposures resulted in significant differences from the controls. Chlorophyll levels in exposed plants were comparable

to those in the controls (Table 26). The only exposures to show a significant difference from the controls were 18 min exposure at 800 m. In some cases, the exposed plants had lower chlorophyll values than the controls; in others, the exposed plants had higher chlorophyll values than the controls. Even though there was statistical significance, no overt bleaching of the sago pondweed leaves was visible to the naked eye. In addition, no significant differences were noted at any of the exposures at 60 and 120 min, times in which prolonged exposure might be expected to produce bleaching from a combination of sunlight and the obscurant.

Table 25. Biomass (g dry weight per plant) of *S. pectinatus* plants after 48 h exposure to fog oil + graphite followed by a 3 week grow-out period (Aug). Initial biomass = 0.16 g. Four plants were exposed at each distance. * indicates treatment mean is significantly different from control.

Dist. (m)	Fog Oil + Graphite 18 min	Fog Oil + Graphite 30 min	Fog Oil + Graphite 60 min	Fog Oil + Graphite 120 min
-50	2.49	2.31	2.32	2.02
5	1.37*	2.93	2.29	1.81
25	2.15	2.33	3.15	2.18
50	1.60	1.94	2.62	1.63
100	1.55*	2.30	2.12	1.46
250	1.44*	1.75	3.08	1.62
500	1.35*	1.93	2.04	1.81
800	1.38*	No sample	No sample	No sample

Table 26. Chlorophyll content (mg chl. a/g fr. wt.) of *S. pectinatus* leaves after 48 h exposure to fog oil + graphite (Aug). Four plants were exposed at each distance. * indicates treatment mean is significantly different from control.

Dist. (m)	Fog Oil + Graphite 18 min	Fog Oil + Graphite 30 min	Fog Oil + Graphite 60 min	Fog Oil + Graphite 120 min
-50	1.1	1.1	1.2	1.1
5	1.0	1.2	1.1	1.2
25	1.2	1.1	1.1	1.2
50	1.1	1.0	1.2	1.2
100	1.2	1.1	1.1	1.0
250	1.0	1.2	1.2	1.1
500	1.0	1.1	1.0	1.0
800	0.8*	No sample	No sample	

4.2.7.2 *Pseudokirchneriella subcapitata*

The only exposures to show a significant difference from the controls was the 120 min fog oil + graphite (50 m) (Table 26). Unfortunately, in the confusion of sorting samples, we lost the controls for the 60 min fog oil + graphite and therefore could not conduct a Dunnett's analysis for that set of data. However, a visual comparison of the values of all of the fog oil + graphite exposed sites at 60 min with the other control values suggests that there were probably no differences in chlorophyll. The sites which most likely received the highest doses of the obscurants (5 and 25 m) showed no statistical differences from the control values, although there did seem to be a suppression of chlorophyll with 30 and 120 min fog oil + graphite (both 5 and 25 m). No

apparent loss of chlorophyll was visible to the naked eye (even after the samples were filtered onto the filter paper, which typically turn green). In general, it did not appear that the obscurants had a significantly negative effect on *P. subcapitata.*

Table 27. Chlorophyll content (µg chl. a/L) of *P. subcapitata* after 48 h exposure to fog oil + graphite (Aug). Four jars with *P. subcapitata* were exposed at each distance. * indicates treatment mean is significantly different from control.

Dist. (m)	Fog Oil + Graphite 18 min	Fog Oil + Graphite 30 min	Fog Oil + Graphite 60 min	Fog Oil + Graphite 120 min
-50	192.3	184.9	No sample	189.8
5	191.4	166.6	192.1	159.0
25	189.0	153.8	208.8	160.9
50	176.9*	189.2	174.1	139.1*
100	182.6	172.3	186.4	203.2
250	179.6	178.0	191.6	192.6
500	161.0	190.8	163.8	199.9
800	No sample	No sample	No sample	No sample

4.3 Laboratory Testing of Fog Oil Toxicity

4.3.1 Laboratory Test Procedure

In surface injection tests with initial fog oils, the 48 h LC_{50} values for the different oils were variable. The old oil, OFO2 was the most toxic with a mean LC_{50} of 4.8 mg total oil/L water (range 2.9 - 6.1 mg/L), while the mean LC_{50} for OFO1 was 102.1 mg total oil/L water (range of 43.1 - 178.8 mg/L). The NFO had an intermediate toxicity with a mean LC_{50} of 18.7 mg total oil/L (range of 11.1 – 28.9 mg/L). Mean LC_{50} values in the separation tests were much higher than those in surface injection tests. While OFO2 was again the most toxic of the three (48-h LC_{50} = 2,500 mg/L, range = 1,005 - >3,756 mg/L), the mean LC_{50} for this oil was two orders of magnitude higher than its value when organisms had direct access to the oil film. Neither OFO1 nor NFO were toxic enough to generate LC_{50} values in separation tests (LC_{50} > 5,000 mg/L).

In experiments where organisms had access to the water surface, all fog oil formulations and concentrations except the 2 mg/L treatment for OFO1 induced significantly increased numbers of floaters compared to combined controls (Dunnett's test, $p < 0.05$, Figure 20). However, the floater phenomenon was quite variable for all three oils tested and not dose-dependent at concentrations greater than 11 mg/L (Figure 20). Due to the lack of dose-dependence, EC50s (concentration that induces a given effect in 50% of sample population) could not be calculated. The percentage of floaters in a given treatment was, however, strongly negatively correlated with percent survival for all three different oil types. The strongest correlation was for NFO ($R^2 = 0.91$, $p < 0.0001$), but both of the old fog oils had high R^2 values as well (0.67, $p < 0.0001$ and 0.69, $p < 0.0001$for OFO1 and OFO2, respectively).

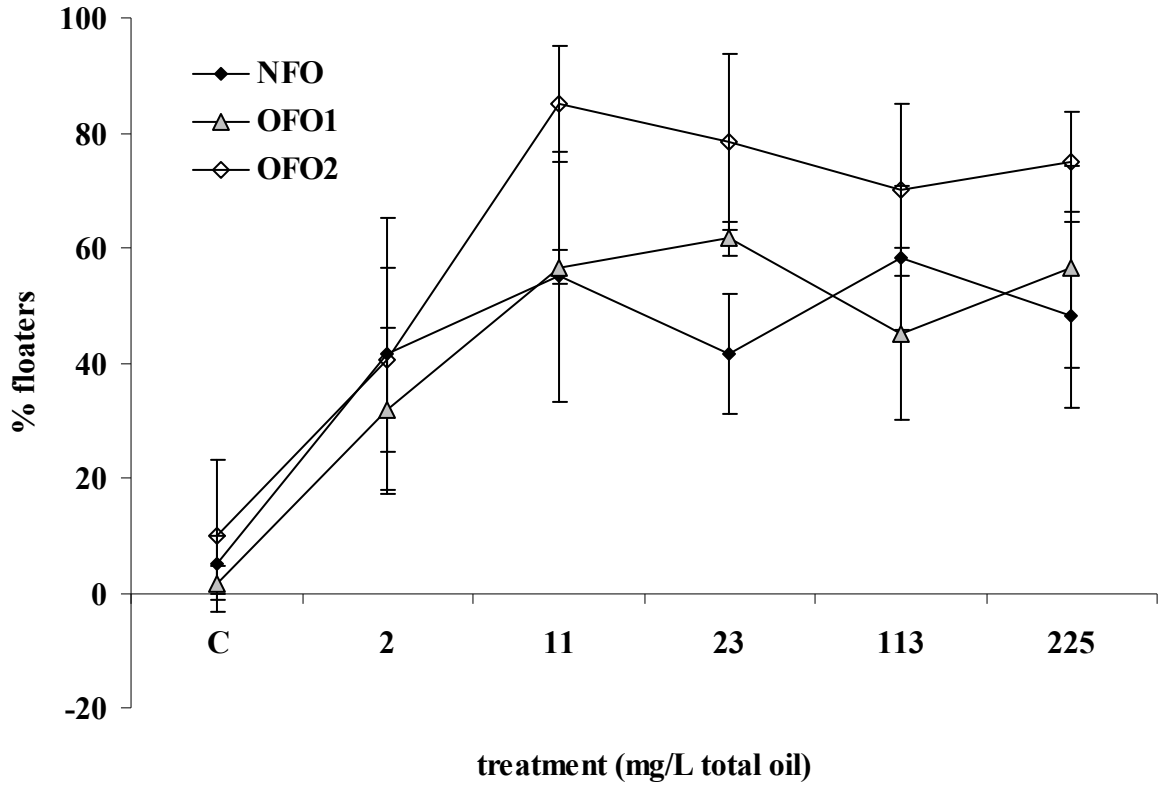

Figure 20. Percentage of floaters in (a) NFO, (b) OFO1, and (c) OFO2 surface injection tests. Vertical bars indicate one standard deviation. The OFO1 2 mg/L treatment was the only treatment mean that was not significantly different from combined controls (Dunnett's test, $p<0.05$).

4.3.2 Generated Versus Initial Oil

In experiments with the generated NFO sample, we observed far less toxicity than when the initial NFO was used. In a side-by-side test, the average LC_{50} with *Ceriodaphnia dubia* for initial NFO was 19 mg/L whereas the average LC_{50} for generated NFO was 1,192 mg/L. In addition, whereas the relationship between % survival and % floaters was highly negatively correlated with the initial oil, there was no relationship (R^2= 0.03) between the two variables when generated oil was used.

In laboratory experiments designed to determine the connection between the floater effect and mortality of daphnids exposed to fog oil, great variation was observed in toxicity of different oils. It is clear that the manufacturing process is not uniform and the designation process ("old" versus "new" fog oil) is broad enough to include oils with widely varying chemical composition. In these experiments, the toxicity of the fog oils followed the progression OFO2 > NFO > OFO1 indicating that newer fog oils were not necessarily less toxic than older oils. However, in all cases, toxicity was substantially decreased when access to the oil film at the water surface was eliminated. In surface injection tests, the number of floaters increased with increasing oil injected up to a point, and then remained relatively constant. In addition, the percentage of floaters was strongly negatively correlated with percent survival for all three oils. These data support the hy-

pothesis of Poston et al. (1986) who concluded that toxicity (mortality) in their laboratory experiments was primarily due to a physical effect caused by the contact of these filter feeders with suspended oil droplets. The chronic effect of this condition is unknown, as is the lowest environmentally relevant concentration of fog oil deposition necessary for occurrence of this effect in the field.

It is not surprising that a fog oil manufactured using the old, less stringent purification specifications would be more toxic than a new fog oil designed to contain less toxic components. Figure 21 illustrates the differences that can arise in different fog oils. HOC is a light yellow oil, a lighter color indicates that much of the aromaticity has been removed as expected for a new fog oil. Surprisingly, the Ft. Irwin oil is even lighter in color despite being manufactured according to the older specifications. AMCO may be quite typical of the older types of fog oil, richer and deeper in color, indicating a higher concentration of aromatic compounds.

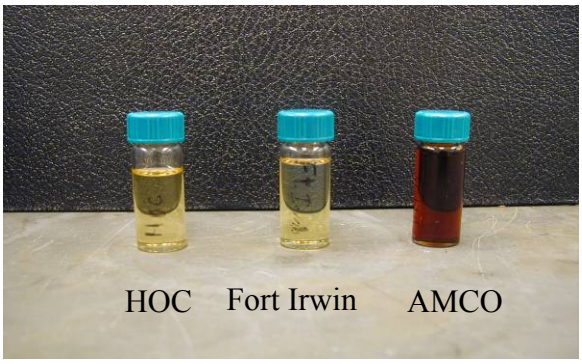

Figure 21. The three fog oils used in experimentation.

Most toxicological research of oil on aquatic species is driven by crude oil spills at specific sites using both the original and the weathered oil. Due to limited mixing and the insolubility of most oil components in water, studies focus on the water-soluble fraction (WSF) or the water-accommodated fraction (WAF) of the original oil. WAF includes both the chemical components of oil that solubilize in water as well as particulates of the bulk oil that enter the water column upon low energy mixing while WSF removes the bulk oil particulates by filtration or centrifugation (Singer et al. 2000, 1998). In either case, toxicity assessment is performed on the oil fraction that enters the water column thereby becoming bioavailable to aquatic biota. Since we did not aggressively mix the sample during the 48 h test period, particulates of oil were not created or introduced into the water column and only the WSF came into play. A separate experiment was conducted to assess the effects of injection of FO at the surface on WSF in MHRW after 48 h. Aliquots of water from beneath the oil layer using the greatest amount of FO injected at the surface (225 µL) had a total organic carbon (TOC) value of 3.04 ppm while the EPA water alone had a TOC value of 2.27 ppm. While TOC is an imprecise measurement of hydrocarbon concentrations, this slight increase in TOC implies that few FO components diffuse into the water column and, furthermore, these water-soluble compounds are non-toxic to the segregated *C. dubia.*

While oil injection experiments demonstrated a relationship between the floater phenomenon and mortality, comparison of generated versus initial oil indicated differences in chemical composition and toxicological responses of *Daphnia*. In fact, in experiments with generated oil, the rela-

tionship between percent floaters and percent mortality observed in experiments with initial oil did not exist. Reasons for this discrepancy are unclear but may again be related to the variability in oil composition. As illustrated in Figure 22, the set of oil constituents in the FO changed upon passing through the generator and condensing on a cold surface. The initial FO clearly lost smaller, more volatile components during the generation of fine aerosol particles. These volatile species remained in the vapor phase and were not observed in the condensed FO. Conversely, larger, less volatile components were more likely to remain associated with the aerosol particles and the recollected FO contained a higher fraction of these compounds in agreement with previous results (Langford, 2004). The sheer complexity of the FO does not permit a detailed identification of the chemical classes that concentrate upon condensation nor does it allow examination of any chemical reactions that may occur upon fog generation.

Additional factors may add to the complexity of the toxic response of aquatic organisms to fog oil. Solar UV radiation (UVR; natural and/or artificially enhanced) and water-soluble contaminants may act either additively or synergistically on a variety of aquatic organisms including amphipods (Ankley et al. 1994; Boese et al. 1998; Diamond et al. 2003), crustaceans (Cleveland et al. 2000; Pelletier et al. 1997; Poston et al. 1988) molluscs (Peachey 2005), and fish (Barron et al. 2005; Bowling et al. 1983; Little et al. 2000). Our field studies documented mortality of and sub-lethal effects to *D. magna* following deposition of fog oil in the field in association with quantifiable residues at very low oil levels. While the NFO used in these field experiments was vaporized, it was not exposed to prolonged solar UV radiation. Thus, they can be considered to be only briefly photooxidized, but not to the degree observed in previous studies of photooxidized fog oil (Poston et al. 1988). It is clear that more research should be done to adequately characterize the risks of fog oil to aquatic invertebrates, including photomodification of FO components on water surfaces. Nonetheless, whereas mortality was observed in our field experiments only at 5 m downwind of the generator, the sub-lethal floater effect was observed up to 50 m downwind. This finding suggests that regardless of the effects of generation or photooxidation on fog oil composition, in the case of daphnids, effects driven by contact with generated oil on the water surface are more important that those driven by the WSF.

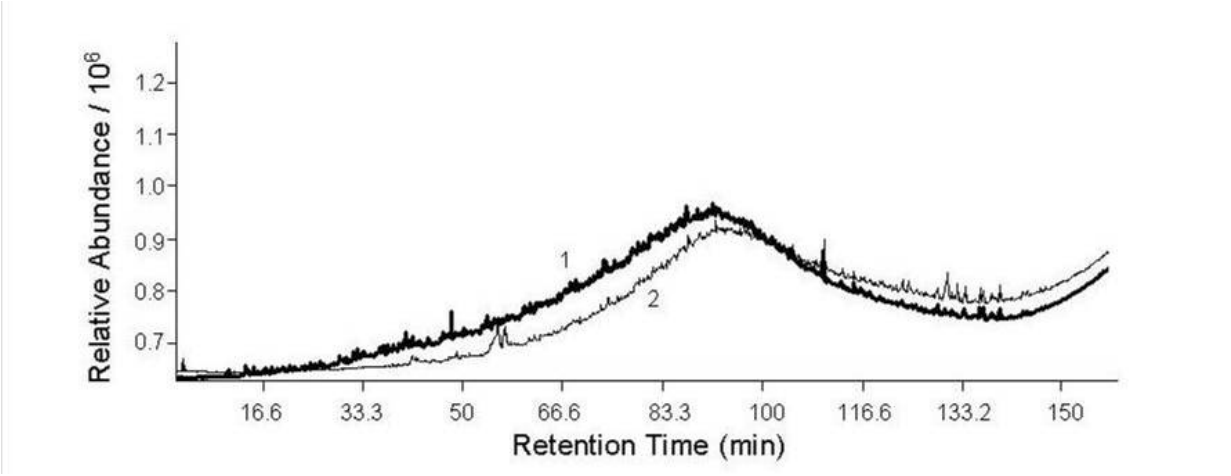

Figure 22. Gas chromatography/flame ionization detection separation of bulk and deposited NFO fog oil. Trace 1 is the chromatogram of initial NFO; trace 2 is the response for a sample of NFO that has been collected at the generator exhaust.

4.4 Laboratory Midge Experiments

Larval survival was high in both experiments with only the high fog oil treatment having % survival lower than 95% (Table 28). This mean was significantly different from the control. In the June experiments, there were no differences among treatments in the number of pupae developing, but mean values of percent survival of pupae, total percent survival, and total percent successful emergence of adults were significantly lower in the high fog oil treatment, relative to controls. There were no differences among treatments in percent partial emergence (adults that attempted to emerge but died while doing so) in either experiment. During the February experiment, there were no differences between the control and the low fog oil treatments, but for every parameter except for percent partial emergence, the means for the high fog oil treatment were significantly lower than both the control and the low fog oil treatment.

Table 28. Mean (± S.D.) % survival, # pupae produced, and % emergence of various life stages of *Chironomus dilutus* in controls and two fog oil treatments from two separate experiments. Low = 1 µl surface injected generated fog oil, High = 100 µl surface injected generated fog oil. Partial emergence = number of adults breaking water surface but not surviving. Means followed by different capital letters are significantly different ($p < 0.05$).

June 2007	Control	Low	High
% survival (larvae)	96 ± 5 A	100 ± 0 A	95 ± 5 A
# pupae	4.0 ± 1.5 A	3.2 ± 1.3 A	4.3 ± 1.5 A
% survival (pupae)	55 ± 24 A	33 ± 37 AB	0 ± 0 B
% survival (total)	78 ± 7 A	76 ± 12 A	53 ± 13 B
% emergence (total)	18 ± 12 A	8 ± 7 AB	0 ± 0 B
% emergence (partial)	6 ± 5 A	6 ± 5 A	0 ± 0 A

February 2008	Control	Low	High
% survival (larvae)	100 ± 0 A	98 ± 4 A	83 ± 13 B
# pupae	6.3 ± 0.4 A	7.3 ± 1.1 A	4.5 ± 0.5 B
% survival (pupae)	67 ± 17 A	64 ± 11 A	13 ± 13 B
% survival (total)	80 ± 10 A	73 ± 8 A	43 ± 4 B
% emergence (total)	43 ± 13 A	48 ± 15 A	5 ± 5 B
% emergence (partial)	0 A	0 A	5 ± 5 A

4.5 Laboratory Assessment of Photolyzed Fog Oil Toxicity

Separate tests were conducted in the fogging chamber on *E. fonticola* adults, larvae, and eggs and larvae. Repeated fogging exposure over the course of several days increased exposure levels and final mortalities were observed. Each fogging event consisted of 2 min of fogging and a 9 h (minimum) settling time. The current study and previous ones have established that this results in FO obscurant air concentrations of 400 mg/min³ (Driver et al. 1993, 2002a, and 2000b; Guelta and Checkai 2001) or greater. This level of obscurance is comparable to that in close proximity to the release nozzle of fog oil obscurant generating equipment during actual field usage.

Pairs of adult fountain darters were exposed to one day of fogging (i.e., one event). The simulated battle field fog oil smoke exposure of the adult fish had no effect on survival of the fish during or after the exposure. All treated and control adult fish survived treatment and the 21 day spawning period. In an attempt to examine an external factor which could have influenced the outcome, standard lengths of individual female fish were plotted against the number of eggs produced by each female. No significant relationship was found. Since no relationship was found, if the random selection of fish for a treatment had ended up with a skewed percentage of the largest fish, this would not have had an effect on the outcome of the trial. Egg production by the adults after the single simulated generated FO exposure also did not appear to be affected (Figure 23 and Figure 24).

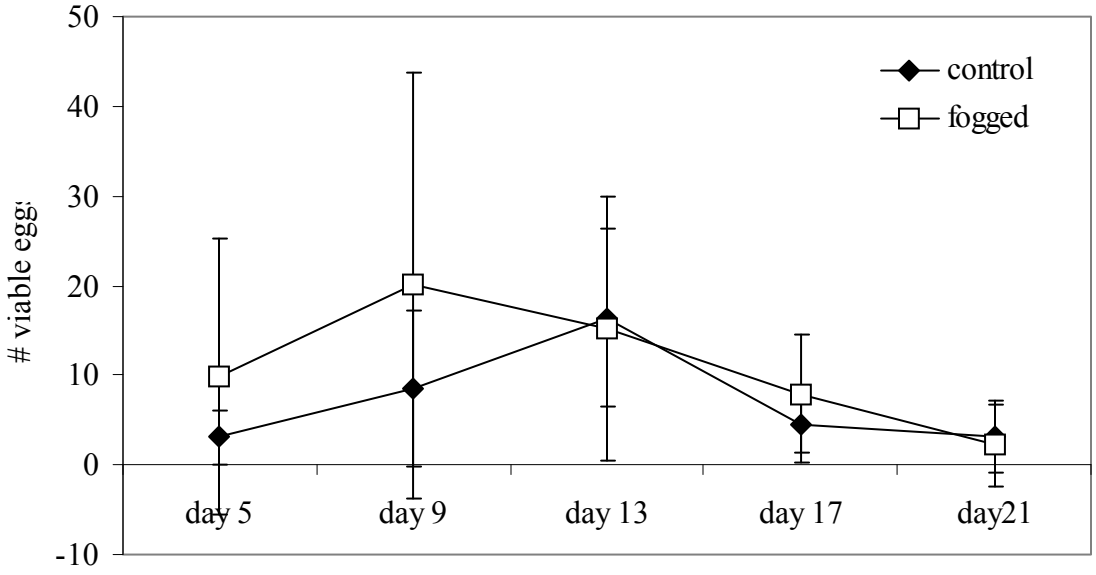

Figure 23. Mean (± S.D.) number of viable eggs produced by individual female fountain darters over 21 days after being either unexposed (control; 24 females) or exposed (24 females) to fog oil smoke. Repeated measures ANOVA indicates no treatment effects (F = 1.546, DF = 22, *p* = 0.2268).

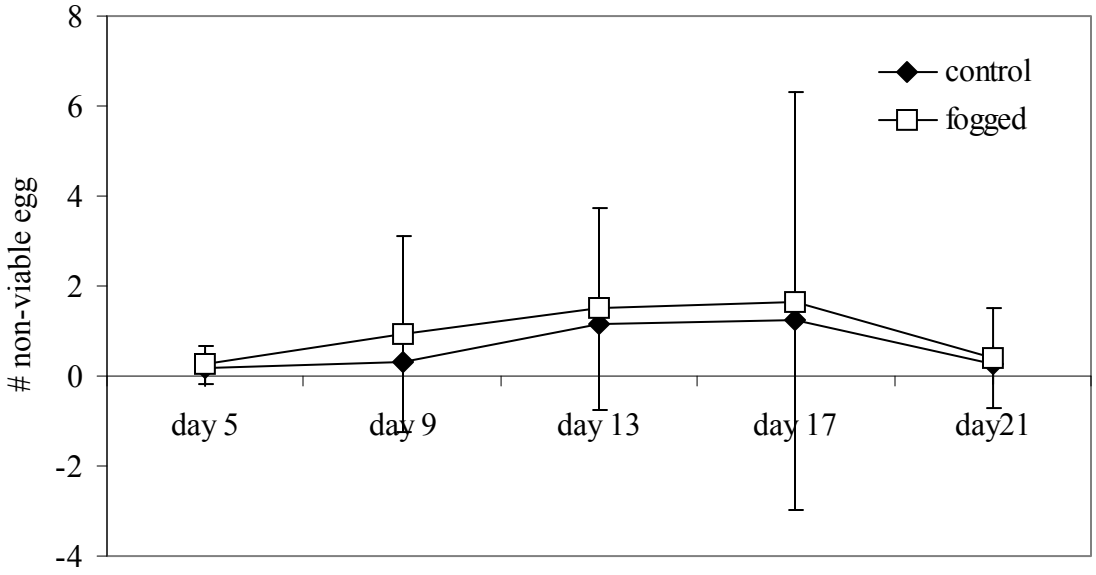

Figure 24. Mean (± S.D.) number of non-viable eggs produced by individual female fountain darters over 21 days after being either unexposed (control; 24 females) or exposed to fog oil smoke (24 females). Repeated measures ANOVA indicates no treatment effects (F = 0.3787, DF = 22, *p* = 0.5446).

Fountain darter larvae, age 72 h post hatch, were subjected to seven consecutive days of fogging. Water was exchanged daily, refogged, and fish were fed *Artemia*. After 5 d of daily generated FO exposures, larvae fountain darter survival was significantly different from the un-exposed larvae. The foggings appeared to have weakened the animals to allow secondary infections of fungus (*Aspergillus spp*.) that cause mortalities in the treated groups. Final survival in the treated groups was 25%, compared to a 55% survival rate in the control group, with treated larva demonstrating an inability to eat, lethargy and death occurring on days 6, 7, and 8.

The effect of daily exposure to FO smoke on larvae fountain darter survival did become significantly different from the unexposed larvae after 7 days. Average survival for two trials went from 85.5 to 60.5 to 36% on days 6, 7, and 8, respectively (Figure 25). Survival for the controls was 97.5%.

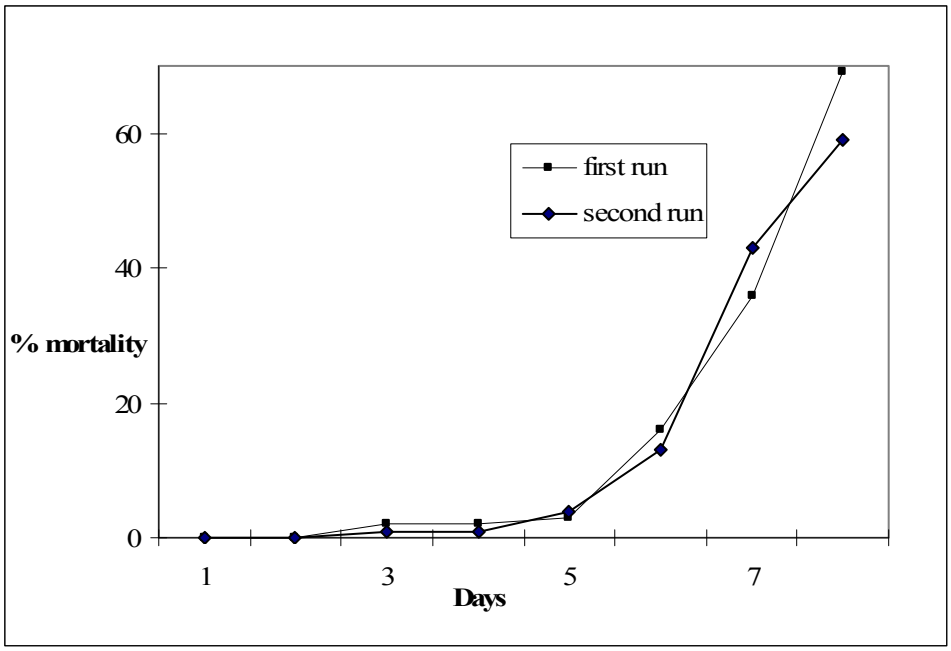

Figure 25. Cumulative mortalities of *Etheostoma fonticola* larvae exposed daily to fog oil smoke for 7 days.

The eggs utilized were 48 h post-fertilization and were fogged daily over the course of three days. Water was exchanged daily, refogged, and non-viable eggs were removed. The eggs were allowed to hatch over the course of the next five days. Results show that survival of the eggs between the control and treatment groups were practically the same: 81% for the controls versus 76% for the exposed groups. Daily exposure of fountain darter eggs to generated FO for three consecutive days did not have an effect on egg survival. Exposures from repeated foggings, however, did affect hatch out rates. Treated eggs hatched at a much slower rate, 6%, than usually observed for untreated eggs, 23%.

4.5.1 Assays for Short-Term Lethality

Fountain darter eggs and larvae were subjected to measured concentrations of generated fog oil in water prepared as water accommodated fractions. Initially, small amounts of fog oil were prepared and mixed into water in an attempt to find concentrations that would induce mortality as chronic 96 h LC-50 exposures. These preliminary range finding tests utilized 1.5, 15, or 150 µL of generated fog oil injected onto 300 mL water shaken by the paint mixer. It was noted that the 150 µL oil addition was the only one of the three that caused some mortality within 96 h. This amount was then used as a base concentration for chronic tests with eggs and larvae.

In an attempt to determine the concentration of fog oil in water as a water accommodated fraction which causes substantial mortality within 96 hours, tests were done on eggs. Over three days, eggs were subjected to five different concentrations of shaken oil/ water mixtures. These treatments utilized 40, 80, 160, 300, or 600 µL of generated fog oil injected into 300 mL of water. None of the concentrations caused complete mortality (100%). The highest concentration of 600 µL (0.2% v/v concentration) lowered survivorship of the eggs by half when compared to the controls (36% vs. 71%), a significant difference in mortality. Other data points are water doped

with 300 μL oil (0.1% v/v) results in 43% survival, 160 μL oil (0.05% v/v) results in 46% survival, 80 μL oil (0.03% v/v) results in 59% survival, 40 μL oil (0.01% v/v) results in 57% survival, and the control (no oil) results in 71% survival. The data show a progressive increase in mortality effects as the oil concentration increases. Preliminary microscopic examination indicates increased arrested development as concentration increases. Secondary fungal infection was also observed to increase with concentration.

Tests using larva show similar results. The survival rates for the larva are as follows: controls had 100% survival, larva in 50 μL oil (0.02% v/v) doped water had a 90% survival rate, 100 μL oil (0.03% v/v) doped water had a 92.5% survival rate, 200 μL oil (0.07% v/v) doped water had a 67.5% survival rate, 400 μL oil (0.1% v/v) doped water had a 12.5% survival rate, and 800 μL oil (0.3% v/v) doped water had a 0% survival rate. It is clear that larvae are more sensitive than eggs or adults to the effects of fog oil in water.

4.5.2 Generated Fog Oil UV Exposure Tests

Control samples with generated fog oil on the surface for 1, 2, 3, 7, and 14 days have TOC levels of 1.7, 2.2, 2.3, 2.8, and 2.6 ppm TOC, respectively. DI water without oil has a TOC level of 0.7 ppm indicating that the generated fog oil has components that slowly enter the water column over time but this stabilizes at about day 3. The samples with generated fog oil at the surface that are illuminated have a drastically different result. Analysis for these samples after 1, 2, 3, 7, and 14 days of illumination are 62, 115, 135, 298, and 439 ppm TOC. UV illumination that mimics solar UV components dramatically increases the amount of oil that enters the water column.

4.5.3 Toxicity Tests with Photolyzed Oil/Water On Fountain Darters

Generated fog oil that has been phototransformed by UV irradiation was demonstrated to acquire increased toxicity due to enhanced water solubility and chemical transformation. Tests were conducted to determine chronic levels of exposure on fish and to compare these results to previous experimental runs involving un-photolyzed generated oil. Solutions of fog oil and water prepared by EPA guidelines were made in uncovered 10 gallon aquaria and exposed to natural UV light outdoors in full sunlight for 3 days. Two batches were produced and analyzed for Total Organic Carbon and pH. TOC values were 38 and 40 ppm, and pH values were 7.0 and 7.8.

With the photolyzed oil/water solution, assays for short-term lethality (96 h, LC_{50}) were conducted on two life stages of fountain darters. Larvae, age 48 hrs post-hatch from egg, and juveniles, age 30 day post-hatch, were exposed utilizing protocols established by the EPA (EPA 2002) for chronic toxicity studies on aquatic organisms. For both the larvae and juvenile fountain darters, toxicity of the photolyzed oil increase dramatically between 15 and 20% of the starting solution (Figure 26 and Figure 27) with LC_{50} values for both life stages at ~16% of solution.

As expected the eggs show a higher tolerance than do larvae and juveniles with toxicity dramatically increasing at between 35 and 45% solution. The LC_{50} was 41.3% of solution (Figure 28). However, the hatch out rate was dramatically reduced in solutions with 25% and more photolyzed water, thus illustrating a substantial sub-lethal effect.

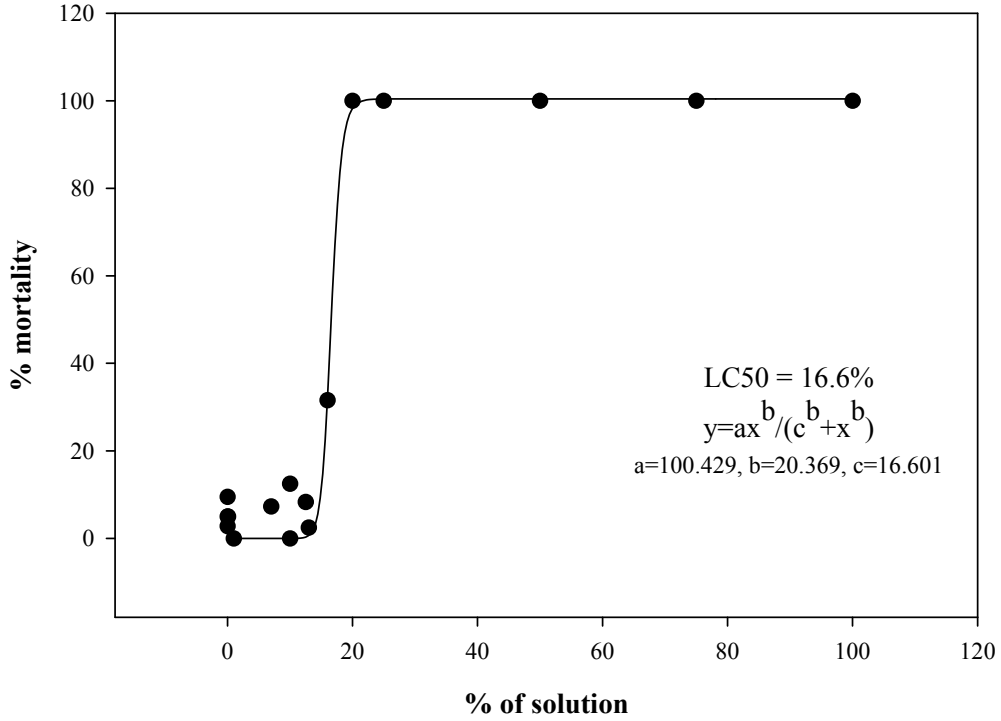

Figure 26. Combined results of three experiments investigating the toxicity of photolyzed oil/water solutions to larval fountain darters (*Etheostoma fonticola*).

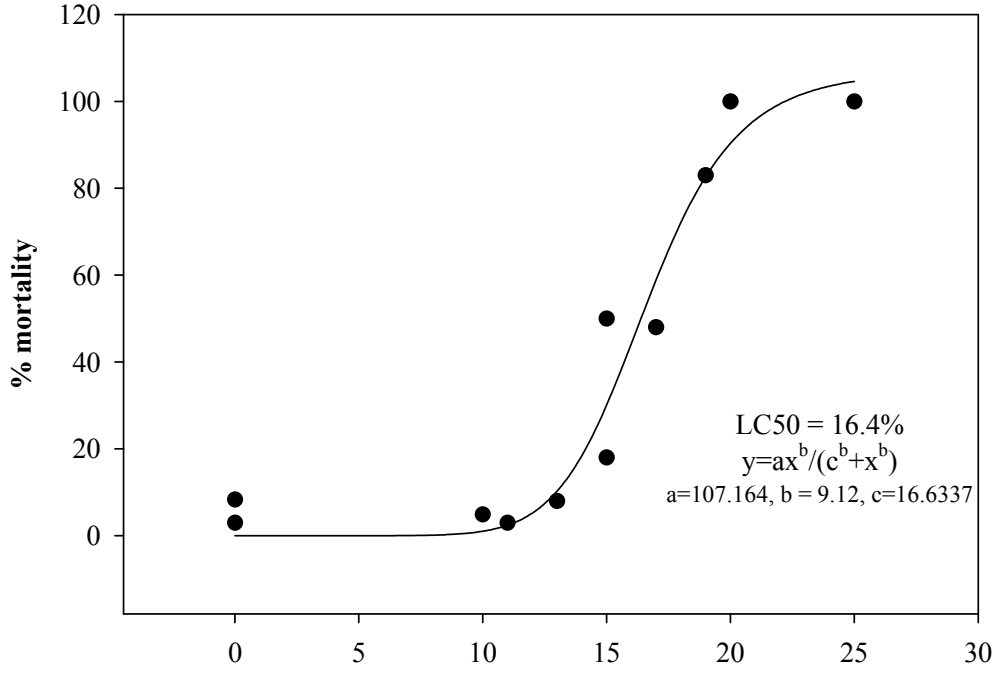

Figure 27. Combined results of two experiments investigating the toxicity of photolyzed oil/water solutions to juvenile fountain darters (*Etheostoma fonticola*).

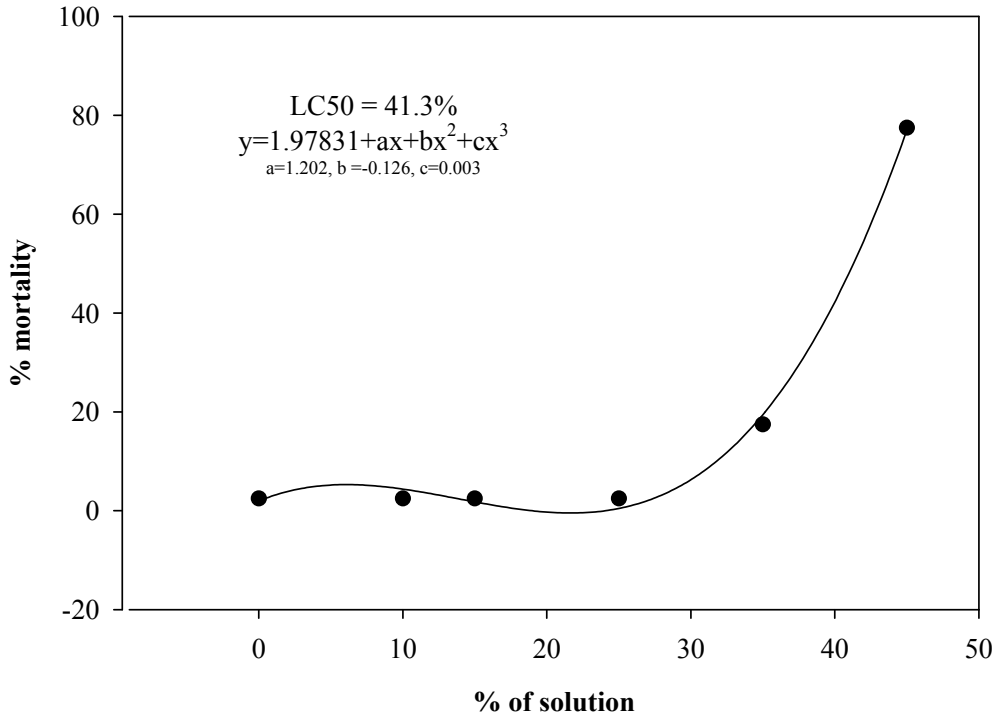

Figure 28. Percent mortality of fountain darter (*Etheostoma fonticola*) eggs in solutions containing various amounts of photolyzed fog oil.

Whereas the water for the above experiments was produced by injecting generated oil onto water and subsequent exposure to sunlight, another approach was made in an effort to better simulate field conditions. Two 15-gallon aquaria were filled with EPA water and exposed to oil fog in a chamber. The fog was produced by the generator and fed into the chamber for 2 min and allowed to settle for 9 hours. The aquaria were then exposed to natural sunlight for 4 days. Quantitative analysis of the oil layer on the water surface showed an oil deposition of 0.1 and 0.15 mg/cm^2. The Total Organic Carbon (TOC) values in the water were 2 and 2.5 ppm.

The fogged and photolyzed water were used for preliminary toxicity tests on juveniles and adult Fountain darters. Concentrations of up to 50% of this water diluted with EPA water did not yield a significant lethality. More fogged and photolyzed water was produced to test its toxicity on fountain darter larvae. This time, the water was fogged 4 times within 2 days and exposed to sunlight for 4 days. The TOC level was 3.1 ppm. No significantly increased lethality was observed in 100% solutions compared to EPA water. This data proves that photolyzed fog oil is not extremely toxic at environmentally relevant deposition levels.

4.5.4 Toxicity Tests with Photolyzed Oil/Water on C. dubia

The 14 day illuminated water was used in a dose response curve test using *C. dubia*. A control of fresh EPA water is also used in the test. After twenty-four hours, 0% of the organisms in the photolyzed water had survived. The organisms in both the fresh EPA water and the EPA water that was exposed to UV and tubing had a 95% and 100% survival rate, respectively. Clearly, the

fog oil undergoes phototransformation to create water soluble components that can kill *C. dubia*. The photolyzed oil components also acidified the water from pH 7.8 to 4.

Water samples drawn from photolyzed water were analyzed by 2D Gas Chromatography after solid phase extraction. The results show that the water contains a spectrum of organic components similar to that of the original oil but shifted toward a higher polarity (Figure 29). Although the chemical structure of the contaminants could not be identified due to the high number of contaminants present in very low concentrations, the results indicate that oxidation reactions of the oil components has occurred to increase the organic contaminants in the water column.

This explains their higher water solubility compared to the original oil and thus the previously observed increase in TOC levels in "photolyzed" water. According to literature, UV exposure of oil in the presence of oxygen leads to oxidation reactions of the oil components. Hydroxyl, carbonyl and carboxylic groups are added, which make the components more polar and water soluble.

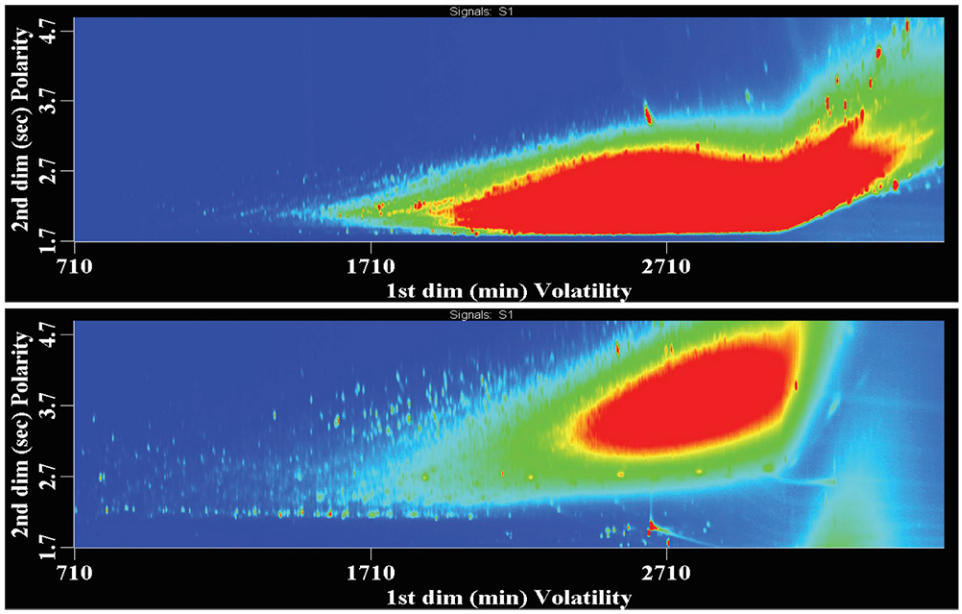

Figure 29. 2DGC analysis of generated for oil and photolyzed fog oil. Top: recollected generated fog oil. Bottom: extract of water exposed to fog oil and sun light.

4.5.5 Generated FO WAF

The greater sensitivity of fountain darter larvae as compared to fountain darter eggs to FO smoke is clearly seen in the LC_{50} values obtained after the fish were exposed for 96 h to generated FO WAF. The threefold difference in 96 h LC_{50} for the larvae (709.4 mg/L; Table 29) is significantly different from the 96 h LC_{50} for the eggs (2105.2 mg/L).

Table 29. The effect of concentration of generated fog oil water accommodated fraction on the survival of *Etheostoma fonticola*.

Life Stage	Fog oil concentration		96 Hours		LC_{50} (95% CI)
	(mg/L)		Mortality (%)		(mg/L)
Eggs	2400		72.5		2150 (2048-2257)
	2025		40.0		
	1650		35.0		
	1275		25.0		
	900		10.0		
	0		7.5		
Larvae	2400		100.0		709 (613-821)
	1200		87.5		
	600		32.5		
	300		7.5		
	150		10.0		
	0		0.0		

4.5.6 Photo-oxidized FO water soluble fraction

The increased sensitivity of larvae fountain darters as compared to eggs was demonstrated again when both were exposed to photo-oxidized FO water soluble fraction (Table 30). The LC_{50} for eggs (40.3% concentration) was over twice the amount needed to kill 50% of the larvae (16.0% of concentration) and the difference was significant. The LC_{50} for juveniles, 17.1 % of concentration, was not significantly different from the LC_{50} for the larvae but it was significantly different from the eggs.

Table 30. The effect of concentration of photo-oxidized fog oil water soluble fraction on the survival of various *Etheostoma fonticola* life stages.

Life Stage	Exposure		96 Hours		TOC		LC_{50} (95% CI)
	Concentration (%)		Mortality (%)		(mg/L)		(%)
Eggs	45		77.5		18.0		40.31 (38.86-41.82)
	35		17.5		14.0		
	25		2.5		10.2		
	15		2.5		6.0		
	10		2.5		4.2		
	0		2.5		0.4		
Larvae	19		100.0		8.7		16.04 (15.48-16.63)
	17		65.7		7.8		
	15		31.4		6.9		
	13		16.7		6.0		
	11		13.9		5.1		
	0		5.6		0.8		

Life Stage	Exposure Concentration (%)		96 Hours Mortality (%)		TOC (mg/L)		LC_{50} (95% CI) (%)
Juveniles	19		82.2		9.6		17.10 (16.56-17.66)
	17		47.5		8.6		
	15		17.5		7.4		
	13		7.5		6.8		
	11		2.5		5.5		
	0		2.5		0.4		

In comparing the sensitivity of the life stages, consideration has to be given to feeding of brine shrimp to the larvae and juveniles during the toxicity trials. Cropek et al. (2008) felt that daphnid mortality was more influenced by physical contact of the organisms to the surface film of oil on the water in the test containers that the actual toxicity of the oil. A surface film of oil was observed on the water of jars during the 7 d exposure of the larvae to the generated FO and on the water in jars containing the higher concentrations of the generated FO WAF. A portion of the brine shrimp consumed by the fountain darter larvae and juveniles probably had come in contact with oil droplets which would mean an additional avenue of oil into the fish through their digestive tract. Determining the route of trophic transfer of FO was beyond the scope of this study.

Analysis of Fog Oil

With the conventional column set, a 5 ˚C temperature difference between the primary and secondary ovens was sufficient to elute all components from the second dimension in the selected modulation time of 5 s. Under these conditions, however, the inverse set led to severe wraparound. Non-polar FO components elute at a lower temperature from the polar primary column then they would from a non-polar primary column (Tran et al. 2006). These non-polar components are then retained strongly on the non-polar secondary column. A temperature difference of 30 ˚C was needed to ensure that the non-polar FO components are eluted within the selected modulation time. In this case, even with a large 30 ˚C temperature difference, the modulation time had to be increased to 7 s to avoid substantial wraparound effects.

A slow temperature ramp rate of 1.5 ˚C/min for both column sets led to maximum use of the separation space for the FO components as seen in Figure 30 during analysis of NFO1. The enormous number of components in NFO1 still overwhelms the separation power of GCxGC-FID as evidenced by the lack of resolution in the bulk of oil signal. Baseline separation occurs only at the fringes of the bulk oil spot for either column set. The alkanes appear in both chromatograms as the typical regularly spaced peaks that have low retention in the polarity dimension. These are identified on the figure through comparison with the standard n-alkane mixture. It is determined that linear alkanes in NFO1 range approximately from C10 to C40 with the main fraction being C16 to C24. NFO1 also contains phytane and pristine; identified through comparison with the standards (Figure 30a). The conventional 2D separation seen in Figure 30b and the inverse separation of Figure 30c both show the characteristic group type bands that are usually observed in petroleum analysis by GCxGC-FID. Alkylated cyclohexanes are more polar than alkanes and are

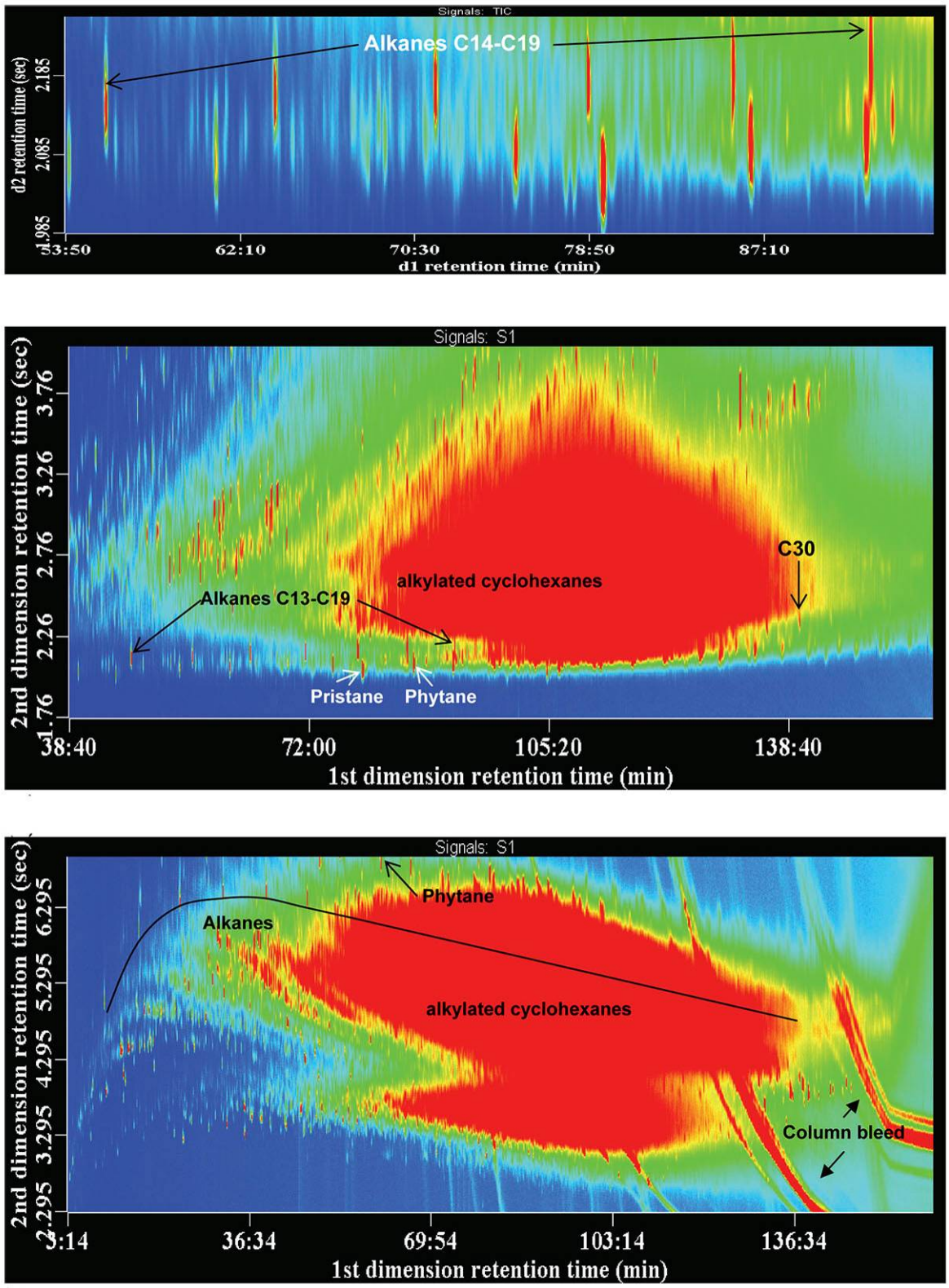

Figure 30. Color contour plot of the two dimensional chromatogram for NFO1 using both column sets and the slow temperature rate. Top: expanded display of the n-alkane region C14 to C19 using the conventional column set. Middle: entire two dimensional separation plane using the conventional column set. Bottom: entire two dimensional separation plane using the inverse column set.

more retained on the polar column. Based on the behavior of the standards, these compounds elute within the unresolved hump and can therefore not be assigned to discrete peaks but position of the band is noted on each figure.

Alkenes elute earlier in the volatility dimension and later in the polarity dimension than their respective alkanes. Alkynes are retained stronger in both dimensions. The few selected alkenes and alkynes that were analyzed elute in the still unresolved complex mixture preventing assignment to a specific peaks. Thus the information gained from this analysis is not sufficient to determine whether these chemical classes are present in the oil. Single ring aromatic compounds and C10-benzenes are more volatile than any oil components and elute earlier first dimension of Figure 30b than the oil hump (not shown). These compounds are not present in any of the FO tested here.

The inverse column set shows the typical problem of column bleed (Figure 30c) which runs across the two-dimensional plane. The pattern, however, is completely different than the conventional separation. Instead of the single oil spot, the chromatogram now consists of two parts, separated in the volatility dimension where the upper part dominates the space. The highly branched alkanes show the highest second dimension retention time of all oil components, with the pristine peak lost from wraparound. The alkanes elute earlier in the second dimension and are denoted by the black line, where longer chain length is retained less in the volatility dimension. The alkylated cyclohexanes are less retained on the second column and analysis of the standard mixture shows the same trend of decreased second dimension retention time with increasing side chain length. Alkenes, alkynes and the alkylated cyclohexanes all elute within the upper section of the still unresolved oil hump. Assignment of any of the standard compounds to specific peaks in the oil was not possible with this separation. This analysis alone but it must be composed of components more polar than the ones present in the standard solutions analyzed.

GCxGC-FID analysis of an old fog oil, OFO2, using both column sets is shown in Figure 31 Using the conventional column set (Figure 31a), a substantial fraction of OFO2 shows wraparound effects indicating a higher amount of polar components than the new FO. The highly branched alkanes like phytane and pristine are identifiable and resolved but the linear alkanes are either present here in small concentrations or co-elute with other components such that they are difficult to specifically highlight in this old FO. Unlike the NFO1 above, n-alkanes in OFO2 span C14 to C26. Most distinctive of this oil is one large peak with low retention in the polarity dimension which, based on the retention behavior of standards, must be a highly branched C20 compound.

With the inverse column set (Figure 31b), OFO 2 shows a similar structure as NFO1 consisting of two large spots on this contour plot. This view clearly shows the gross differences between a new and old FO. While the upper spot outweighs the lower spot in NFO1, these spot are approximately equal in size in OFO2. This confirms that the old FO contains a higher amount of more polar, unsaturated components than the new FO. In this inverse configuration, the highly branched alkanes can be seen at the top edge of the upper spot.

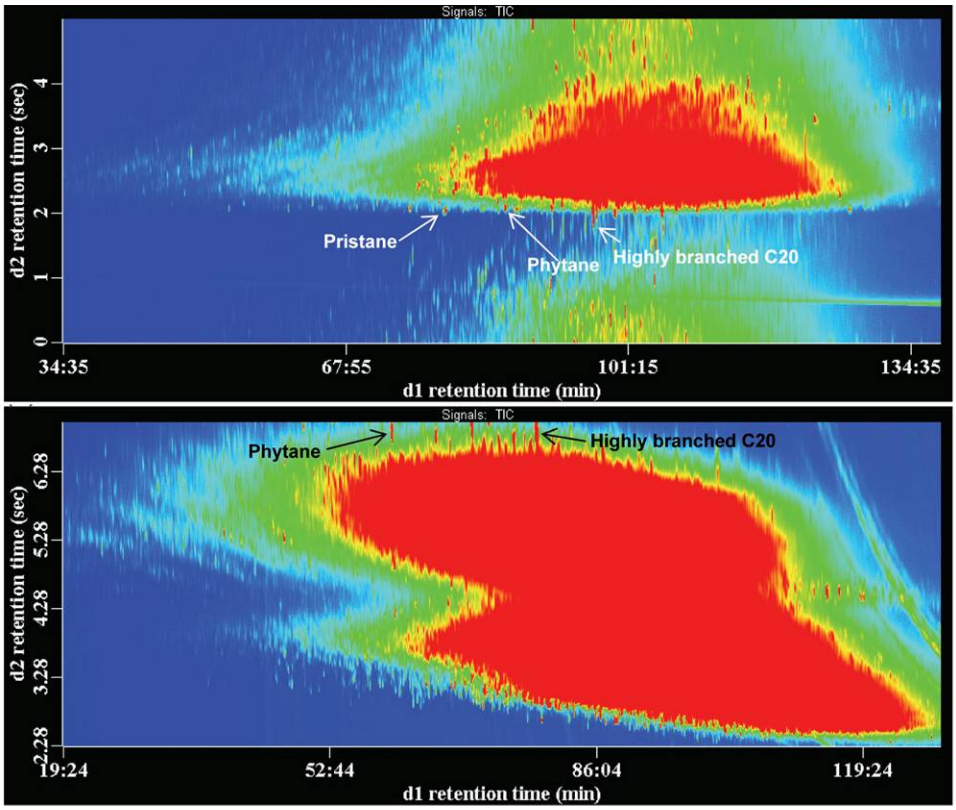

Figure 31. Color contour plot of the two dimensional chromatogram for OFO2 using both column sets and the slow temperature rate. Top: entire two dimensional separation plane using the conventional column set. Bottom: entire two dimensional separation plane using the inverse column set.

4.5.7 Silica Gel Extraction

The fractionation procedure was tested using a combination of all standards in one mixture, including the n-alkanes (C9-C36), alkylated cyclohexanes, alkenes, alkynes and PAH. The results followed the expected fractionation except for the alkynes. The saturated compounds all eluted in F1.1, the alkenes in F1.2 and the PAH in F2. Although expected in F1.2, alkynes were not recovered in any of these fractions and must be retained too strongly on the silver impregnated silica gel.

This silica gel separation was performed on NFO1 and OFO2. Ten μL of oil was dissolved in n-hexane and loaded onto the silica gel. Shown in Figure 32 and Figure 33 are the fraction chromatograms for NFO1 and OFO2, respectively, using the inverse column set with F1.1 (top), F1.2 (middle) and F2 (bottom). As expected, NFO1 mainly consists of saturated compounds which elute in F1.1. Surprisingly, fraction F1.2 shows a substantial presence of components although these new oils are subjected to a refining process designed to remove unsaturated hydrocarbons. A number of evenly spaced, well separated peaks at a higher second dimension retention time are the linear, even-numbered alkenes (C14 to C22) with one terminal double bond, based on comparison with standard solutions. F2 can contain aromatic compounds, but it is more likely that this fraction is composed of non-aromatic polar species. This F2 band overlaps with the F1.2 band and may contain identical compounds due to non-optimal fractionation, but it is noted that

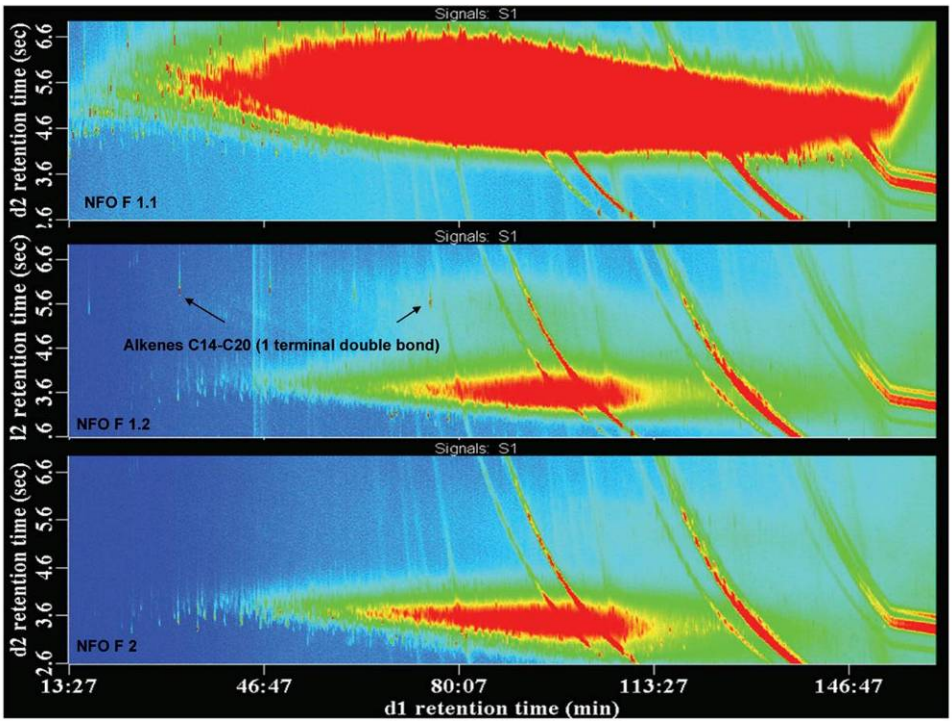

Figure 32. Color contour plot of the three fractions after silica gel separation of NFO1 using the inverse column set. Top: fraction F1.1. Middle: fraction F1.2. Bottom: fraction F2.

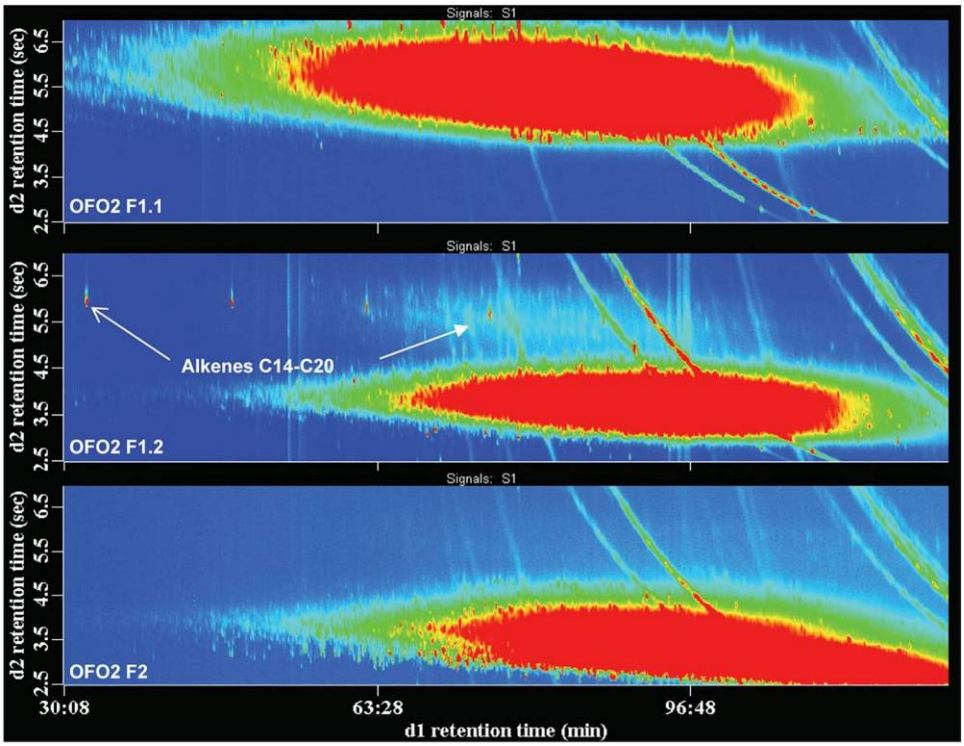

Figure 33. Color contour plot of the three fractions after silica gel separation of OFO2 using the inverse column set. Top: fraction F1.1. Middle: fraction F1.2. Bottom: fraction F2.

the alkenes are not present in F2. Silica gel fractionation followed by analysis with the inverse column set allows partitioning of the complex FO mixture into saturated compounds and unsaturated polar compounds. With the conventional column set, these fractions overlap so that a spatial differentiation of the oil hump into chemical classes is not possible (not shown).

The three fractions of the old oil OFO2 are shown in Figure 33 Like NFO1, the double lobed structure observed using the inverse column set on the entire oil (Figure 31b) is separated into its two parts by silica gel fractionation. Clear differences between the relative sizes of the nodes for NFO1 and OFO2 are distinguishable; these fractions are all approximately the same size for OFO2. Again, the linear alkenes are seen as discrete peaks in F1.2. The population of aromatic and polar compounds in F2 is substantial in OFO2. The post-processing steps for refining new fog oils is successful in decreasing a large proportion of the unsaturated and aromatic constituents as evidenced by the much smaller F1.2 and F2 nodes in the NFO1 analysis.

4.5.8 Polycyclic Aromatic Hydrocarbon (PAH) Analysis

Polycyclic aromatic hydrocarbons (PAH) are a family of organic compounds that consist of a number of fused aromatic rings (generally more than 2) that are widely known as some of the more potent contaminants present in carbonaceous deposits such as oils, tars and coals. Since FO are released on training grounds during soldier maneuvers, it is critical to ensure that carcinogenic, teratogenic, and mutagenic PAH are not present within the oil for human health protection and environmental compliance. To verify that PAH could be identified in FO if present, NFO1 was spiked with the PAH standard at 55 μg PAH / g FO. PAH are common contaminants in oils and their determination in a complex oil matrix usually involves labor intensive sample preparation which increases the risk of analyte loss. Instead of separating PAH from the matrix in a pre-analysis clean-up step, two dimensional chromatography was investigated for the ability to separate these aromatic constituents in the two dimensional plane. Due to the higher polarity of PAH compared to most oil components, they elute at a high second dimension retention time on a conventional column set. To avoid wraparound, the PAH method described above employs a fast temperature program of 5 ˚C/min and a 10 ˚C temperature difference between primary and secondary ovens. Under these conditions, the oil components are elute within a small band at a relatively low second dimension retention time, whereas PAH elute just above the oil hump. Figure 34 (top) depicts a region of the NFO1 chromatogram using this PAH method on the conventional column set and Figure 34 (bottom) shows the same region for a sample of PAH spiked NFO1. The figure clearly demonstrates that this method can separate these 3, 4, and 5 ring PAH from the complex oil hydrocarbon mixture. The NFO1 does not contain any of these PAH. All PAH are baseline separated from another and are easily detectable, PAH with molecular mass above 276 will wrap around and elute before the oil hump (not shown). Using this method, none of the four military fog oils contained PAH peaks. Further manipulation of GC parameters would likely avoid the wraparound effects for the higher PAH.

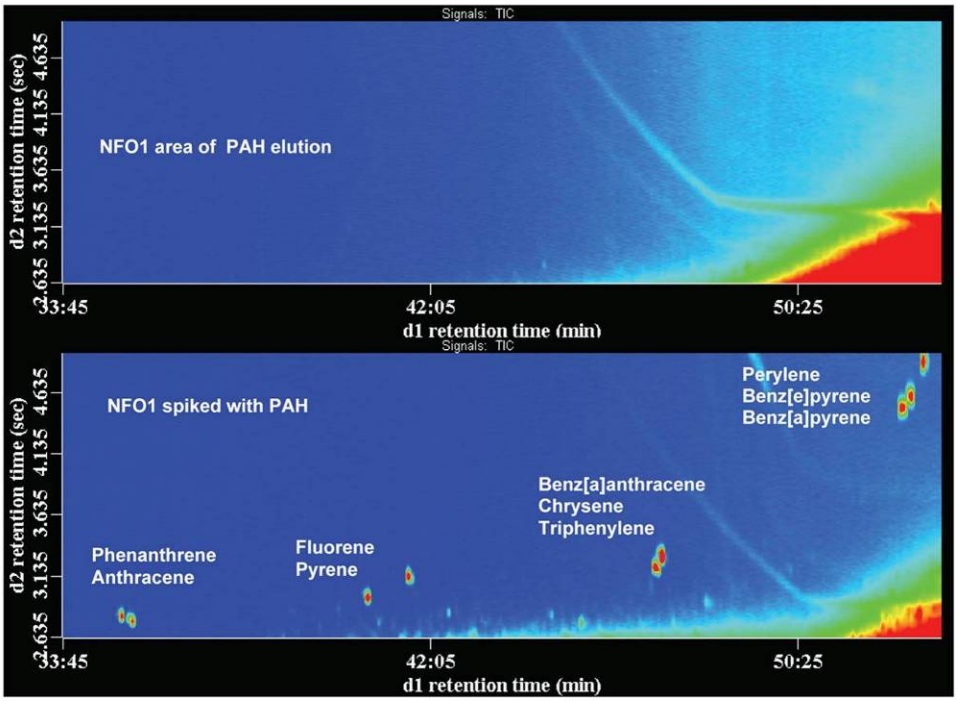

Figure 34. Color contour plot of the PAH elution area of NFO1 using the conventional column set. Top: NFO1 alone. Bottom: NFO1 spiked with PAH.

4.5.9 Distinguishing oils

With either column set, a fast temperature rate of 5 ˚C/min yielded sufficient resolution to distinguish a new and old oil type from one another and to separate hydrocarbon oil components from carcinogenic PAH in analysis times of 86 or 102 min, respectively. Four different military fog oils (two new and two old ones) were analyzed to test the conventional column set method to distinguish several FO from each other. The surface plots for NFO1, NFO2, OFO1, and OFO2 are shown in Figure 35a – d, respectively. These surface plots are angled to view along the second (polarity) dimension and, even from a distance, this view proves instructive to fingerprint each of the four oils.

The white lines on the left side of each chromatogram denote the first (volatility) dimension, i.e., the typical unresolved complex humps (UCH) seen in one dimensional gas chromatography. There are only slight differences in these UCH which are insufficient to serve as identifying markers for oil type.

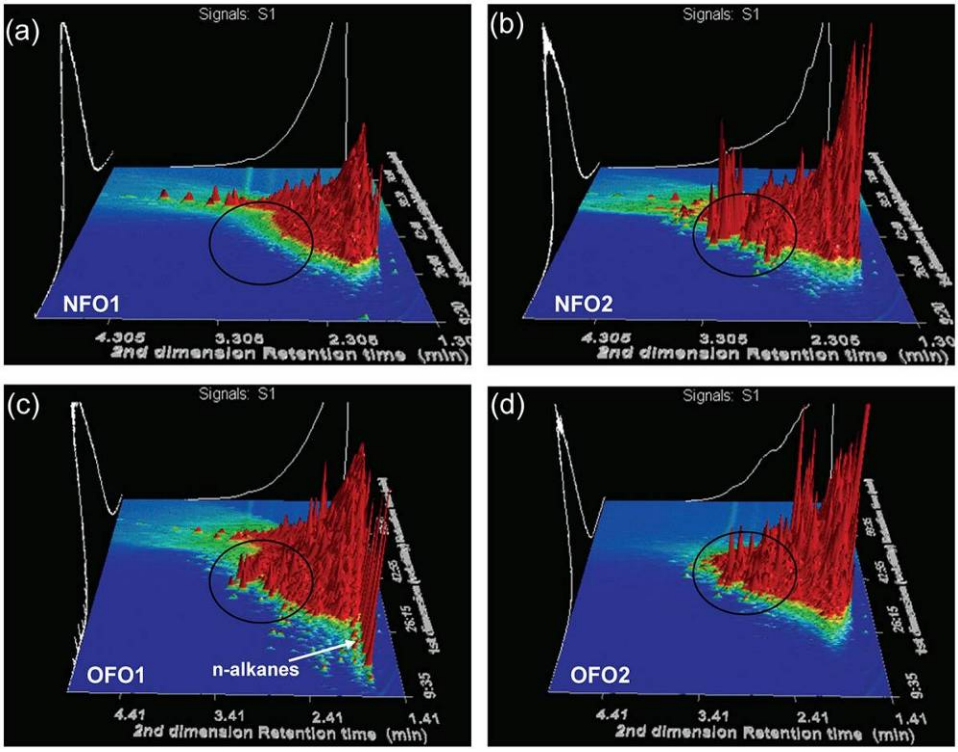

Figure 35. Color surface plots of the four different FO using the conventional column set. (a): NFO1. (b): NFO2. (c): OFO1 (d): OFO2. Circled regions indicate the elution space of higher polarity and aromatic compounds. Additional n-alkane compounds are indicated by the arrow on OFO1 (c).

As Figure 35 demonstrates, these surface plots show a distinctive pattern for each oil that renders the oils distinguishable from each other. All oils have their main fraction at low second dimension retention times due to alkanes and branched alkanes but differ in the amount of components eluting at higher second dimension times. The additional processing of oils after 1986 changes the population of oil components with longer retention in the polarity dimension, this region of each oil chromatogram is denoted by a black circle on the figures. NFO1 has fewer components present within the circled region and represents the cleanest of the oils in this study. While NFO2 is labeled as a new FO, it clearly has a substantial presence in the processed region which may indicate insufficient processing or contamination. Both OFO1 and OFO2 have many compounds that elute in the processed region as expected but differences in between these two oils are easily seen just in this region. OFO1 also shows a more extensive n-alkane population than any other oil. The major branched C20 hydrocarbon of OFO2 stands out in this chromatogram. These patterns give each oil a unique fingerprint that can serve as identification, ensuring adequate cleanup of oils, and possibly distinguishing these FO from natural oils once present in the environment.

4.6 Other Items and Issues

4.6.1 Toxicological Difficulties

Although we make clear that observed mortality or other effects was generally non-existent or low for all test organisms, experiments with oils, and fog oil in particular, is extremely difficult.

First, FO consists of millions of components and these differ based on manufacturer, batch to batch, and lot to lot. This is not a typical toxicity test scenario where a single chemical component is the target. Even knowing the toxic effects, these cannot be related back to a single chemical or even to a class of compounds. Further, hydrocarbon oils are non-water soluble. Therefore, we cannot perform standard toxicity measurements where a known amount of chemical is dissolved in water. The amount of oil that enters the water column is not controllable and the dissolution process fractionates the oil into classes. Finally, aquatic organisms can experience both the water soluble fraction and the concentrated oil film at the water surface due to water motion within the mesocosm. Additionally, wind, hydrologic flow, and temperature induced mixing may cause some dispersion of oil into the water column. This will serve to combine effects due to bioavailable oil compounds in the water with a concentrated direct dose at the surface.

Second, not only is the set of complete chemical constituents of FO unknown, transformation of these chemical constituents as a result of the combustion processes used in the generation of S&O is also unknown, and the subset of these constituents that deposits at the water surface renders any definitive listing of chemicals at the water surface virtually impossible. In addition, sensitivity to photolytic processes, volatility, and biodegradation serve to complicate toxicity analysis.

Third, air temperature, relative humidity, and/or general climatologic conditions play a role. Exposure test starting times varied from 0600 hrs to 1700 hrs and ambient air temperatures varied from 25 °F to 55.5 °F to 70 °F. Effects of air temperature and other climatological factors on S&O deposition are uncertain. The exposure events produced were intended to be representative of multiple climatological conditions, typical of actual military field training usage.

Fourth, the variable nature of wind and its direction and velocity confound efforts to obtain uniform exposures and associated deposition. Some of the S&O appeared to loft or rise more than others due to air circulation. Clearly this varying and uncontrollable factor will have a drastic effect on the ability to perform reproducible deposition testing in the field.

In the first year of this research effort, data was obtained from effect testing during field release of colored smokes at Aberdeen Proving Ground, MD. Those tests involved exposure of several trophic levels and phylogenetic groups of test organisms (i.e., plant, invertebrate, vertebrate) to concentrations of green, yellow, and red smoke. The outcomes of those preliminary studies required the release of a smoke grenade onto a microcosm placed one m from the release point to have any detectable negative (i.e., toxic) effect on at least some biological levels. Toxicity was apparently was due to heavy deposition of the dye particulates onto the water surface at this extremely close range. Since there is a dearth of data for effects of deposited colored smoke dyes and particulates on ecologically relevant plant or animal species, however, we continued to pursue acquisition of colored signal smoke grenades for more in depth laboratory investigation.

From early onset, obtaining supplies of the M18 grenades proved to be difficult, apparently attributable to war time contingencies. This issue was addressed in a previous White Paper (dated September 2005). In March 2007, however, we obtained 20 green, 20 yellow, 20 red, 10 orange, 10 violet, and 10 blue signal smoke grenades for research on potential toxicity effects. These grenades represent the currently approved chemical formulations for these colors and are the

grenade types in training use. Significant quantities of these formulations exist and will continue to be used until those supplies are exhausted. It is our understanding that newer formulations of at least some colors of signal smoke grenades (primarily using sugar [e.g., C_{12} $H_{22}O_{11}$] as a fuel source instead of sulfur, and $MgCO_3$ [magnesium carbonate] instead of $NaHCO_3$ [sodium bicarbonate] as a coolant) are being developed and tested but are not yet in common use. Furthermore, these new formulations have not yet been assessed for their "environmental toxicity". Due to the late arrival of these colored smoke grenades and the lack of field toxicity, it was recommended to us by SERDP that we eliminate further experimentation with these smokes.

4.6.2 Effects of S&O on Freshwater Mussels

This objective and associated tasks was deemed superfluous based on results of early experiments. Our field and laboratory results determined that (a) only marginal acute toxicity was observed in *Daphnia* (a sensitive test organism) tested in the field and there was no toxic effect on any other higher order organism or plant in our studies. (b) Both laboratory and field experiments indicated that toxicity and/or sub-lethal effects were associated with or directly caused by organisms coming in contact with the oil in surface film. (c) Dissolution of fog oil components into the water column is minimal although this increases with prolonged weathering. Freshwater mussels are not only benthic, but also sessile, and therefore, the likelihood of these organisms coming in contact with fog oil and thus experiencing toxic effects is highly unlikely.

4.6.3 Sublethal Effects Observed

While acute toxicity (i.e., mortality) due to fog oil exposure was observed during this study, sublethal effects dominated, and in some cases, the sub-lethal effect eventually resulted in mortality. These sublethal effects were not necessarily related to toxicity per se, but rather to what might be characterized as physical interaction or physical barrier effect. The first important sub-lethal effect observed was what Poston et al. (1986) termed "the floater effect", i.e., daphnids in chambers exposed to fog oil were much more likely to become stuck in the surface film than were control organisms. Often this effect was associated with higher mortality, and our laboratory experiments indicated that becoming stuck in the surface film was almost necessary to cause mortality. The other major sub-lethal effect also was associated with contact with the surface film of fog oil. Midge (*Chironomus dilutus*) larvae that pupated and then attempted to emerge from the water as adults were much less likely to be successful when a high dose of generated fog oil was applied to the water surface.

4.6.4 Fish Sublethal Effects Data

While acute toxicity (i.e., mortality) to various species and genera of adult fish at field and laboratory simulated exposure levels, was not observed, toxic effects on fish larvae at high mixed fog oil and photolyzed FO were noted. Thus, it is important to consider various life stages when conducting toxicity tests. Similarly, it is important to consider the various exposure possibilities (i.e., generated FO, WAF, photolyzed FO) in conducting testing. Also, our laboratory tests involved one species. Fountain darters have been shown to be more sensitive to interstate highway storm water runoff than other commonly used species such as the fathead minnow. Other species may be more or less sensitive to petroleum derivatives. Sub-lethal effects on fish are few and there are

none when using environmentally relevant concentrations of FO. The presence of larger amounts of FO decreases the hatchout rate of Fountain darter eggs and can weaken the resistance of the eggs to fungal attack.

4.6.5 Plant Effects Data

Exposure of representative phytoplankton and submergent vascular plants to relevant FO obscurant concentrations under field conditions did not produce any observable effect. Although unlikely given the concentrations involved and other work reported on agricultural plant oils, aquatic plants (e.g., emergent species) may respond differently.

4.6.6 Particle Size Distribution Of the Fog Oil

The expelled fog oil vapor condenses upon contact with air and forms a dense, white cloud. The particle size distribution of this FO smoke has been analyzed previously and is actually a mist composed of fine liquid droplets with a mean diameter around 0.8 μm (DeVaull et al. 1989).

4.6.7 Plume Movement

We contacted Dr. Wayne Miller from the University of California–Riverside to discuss plume movement data and congruous sampling techniques. We considered that our field data showed that oil deposition occurred only within the first 50 m of the release point and long range effects, where plume movement data would be important, were non-existent. We also observed that Dr. Miller determined atmospheric movement while we are concerned with aquatic deposition and the two sampling and measurement techniques will be unrelated. While plume movement may be important for air quality and other reasons, given the multiple influences of small aerosol droplet size (DeVaull et al. 1989), photolysis, oxidation, volatilization, and atmospheric conditions, the relationship of plume movement to deposition is probably most closely related to wind and convective air currents. Finally, after the first year of research, we no longer pursued field experimentation making extensive plume movement research unnecessary.

4.6.8 Metal Deposition From the S&O

We had the different oils used in this research analyzed for metal composition by PDC Laboratories (Peoria, IL) a certified laboratory. These results are compiled in Table 31. We note that HOC FO has few metals present, only manganese, selenium, sulfur and zinc are above detection limits. Sulfur in HOC FO is greatly reduced over old FO types (ERDEC, AMCO, Ft. Irwin). The additional processing required for new oils dramatically removed these sulfur containing compounds. Only selenium is a great concern. Comparing initial HOC FO to HOC FO after it has been through the generator shows that some selenium and all zinc and manganese is lost, while sulfur slightly concentrates. Based on our toxicity data, especially the separation experiments with *C. dubia*, these metals do not enter the water column sufficiently to produce a toxic effect.

Table 31. Laboratory results on metal composition of different fog oils.

	HOC FO	ERDEC FO	AMCO FO	IRWIN FO	Generated FO
Aluminum	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg
Antimony	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg
Arsenic	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg
Barium	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg
Beryllium	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg
Boron	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg
Cadmium	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg
Calcium	< 5 mg/kg	< 5 mg/kg	6.6 mg/kg	11 mg/kg	< 5 mg/kg
Chromium	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg
Cobalt	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg
Copper	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg
Iron	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	5.6 mg/kg	< 5 mg/kg
Lead	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg
Magnesium	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	7.1 mg/kg	< 5 mg/kg
Manganese	21 mg/kg	< 5 mg/kg	9.4 mg/kg	< 5 mg/kg	< 5 mg/kg
Molybdenum	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg
Nickel	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg
Phosphorous	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	7.2 mg/kg	< 5 mg/kg
Potassium	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg
Selenium	14 mg/kg	21 mg/kg	< 5 mg/kg	< 5 mg/kg	10 mg/kg
Silver	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg
Sodium	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg
Sulfur	42 mg/kg	6700 mg/kg	4300 mg/kg	2000 mg/kg	63 mg/kg
Thallium	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg
Vanadium	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg	< 5 mg/kg
Zinc	18 mg/kg	< 5 mg/kg	13 mg/kg	< 5 mg/kg	< 5 mg/kg

FO can enter aquatic systems through a variety of routes. These include airborne deposition (including that facilitated by precipitation), airborne deposition onto terrestrial surface with subsequent transport, and direct deposition onto aquatic surfaces (spills). Spills, while possible, are unlikely and only of local concern. FO obscurant deposition on non-aquatic surfaces with subsequent transport or migration to aquatic environments via rain or snow is possible, but beyond the scope of this investigation. Once in aquatic system, FO chemical components are available and subject to various uptake and breakdown pathways that exist. Our work suggests physical contact with FO is an important element of any environmental effect. In lotic environmental system, FO obscurant movement and dilution would be greater than that in standing environments.

Our tests evaluated toxic effects of FO obscurant deposition onto an aquatic environment where the mode of exposure is initiated through airborne deposition. In that sense, exposure is analogous to that of other potential airborne petroleum pollutants. In other work, Douglas et al. (2006) have observed that due to evaporation, absorptive and repellant qualities, and surface chemical properties, collection media can influence FO obscurant deposition measurements. In this work (Cropek et al. 2008), we have established that water is an effective FO obscurant collection me-

dia. Further, our FO obscurant deposition analysis results can be considered representative of those expected under similar military FO obscuration events. Once the FO is present at the water surface, examination of ultimate FO sinks in the environment and the potential routes were beyond the scope of this project.

The similarities of smokes and obscurants produced between the smoke generator used and those used during actual operations.

Our field generator is exactly the same as that used in actual military training operations, therefore, our field smokes were identical to those which arise during training exercises. Our lab generator used the same HOC FO as that used in the field and we operate our generator at the same temperatures to produce the same fog oil clouds. According to the chromatographic data, field generated FO deposition is the same as that we obtained in our experiments.

5. CONCLUSIONS

5.1 S&O Release and Deposition in the Field During Simulated Training Events

Based on the data presented, each type of S&O examined has an optimal filter collection. For green colored smoke, ACF-7 is the best filter to collect the green deposited dye, but it may be difficult and expensive to acquire. For commercial filters, PTFE performs best and would be readily available. It is suspected that ACF-7 would also work well for collection of yellow dye, as green and yellow dye are similar in molecular size and the functional groups are similar. When the sampling area was close to the release point, SB6407 performed best for yellow dye because it had a higher capacity for dye collection. Farther away from the release point, Supor was best for yellow dye because it can collect more dye in dilute conditions.

For FO, the recommendations are slightly more complex. If the goal is to collect the deposited oil fraction that best represents what would deposit onto a water surface, then GFF works well, and it is inexpensive. To collect all the oil that can deposit onto surfaces, Metricel, a hydrophobic polypropylene, works best.

These recommendations will be useful for site characterizations to minimize the amount of materials and equipment needed in the field. Transportation of jars of solvents is eliminated. Solid filters can be placed in difficult to access areas such as tortoise holes or in tree branches to determine the S&O that can deposit in these unusual geometries.

Finally, the need to test each type of S&O deposition independently is recognized. It is strongly encouraged to use an enclosed chamber for controlled release and equivalent deposition for direct comparison of filters.

5.2 Fog Oil and Fog Oil and Graphite Mix

While deposition rates were variable, we measured fog oil deposition on water samples as far as 50 m downwind of the generator. Wide variation of FO deposition rates at 5 m may be attributed to larger droplets emitted from the generator and not a FO aerosol characteristics fog per se. Fog oil obscurant deposits were never detected farther than 50 m downwind of the generator.

5.3 Colored Smokes

Colored smoke particle deposition was greater close to the release point and decreased rapidly beyond 5 m to non-detectable levels beyond 25 m. Only at very close (1 m) distances from the release point did the colored smoke dye contaminate the water enough to cause toxicity to aquatic organisms, and this required water samples in the direct line of the smoke emission.

5.4 Field Toxicity of Fog Oil on Aquatic Organisms During Simulated Training Events

While the varying ambient field conditions pose a number of challenges to acute toxicity testing with FO, our results showed that acute toxicity to *Daphnia magna* was measurable under field

conditions. We observed increased mortality at 5 m downwind from the generator and increased numbers of organisms stuck in surface film as far as 50 m downwind. In experiments by Poston et al. (1986), daphnids were caught in the surface film at concentrations as low as 30 µg total fog oil/L, in agreement with our field experiments. We speculate that this effect may be due to the ingestion of oil microdroplets, which accumulate in the individual and ultimately induce buoyancy. We were unable to detect any acute toxicity to other at other trophic and phylogenetic levels involving green algae, submersed vascular plants, several species and genera of fish, and a common amphibian.

5.5 Laboratory Testing of Fog Oil Toxicity

While several factors, including generation and photooxidation, may change the composition and therefore the toxicity of fog oil, both field and laboratory experiments suggest that physical contact with oils on the water surface is an important factor driving toxicity. This was based on correlations between mortality and number of floaters in *D. magna*. In addition, laboratory experiments explicitly designed to prevent access to the water's surface resulted in LC_{50} values that were at least two orders of magnitude higher than when access was permitted. It is also interesting that our data showed that generated FO is less toxic than the initial FO. This is likely due to the loss of low volatility, low mass hydrocarbons that can be more bioavailable to aquatic organisms.

Fountain darter larvae are more sensitive than adults, juvenile, and eggs to FO. LC_{50} values for darter larvae exposed to FO dispersed in water under laboratory conditions was three times greater than those observed for eggs.

5.6 Laboratory Midge Experiments

Low levels of fog oil deposition (1 µL on a 1 L beaker or ~785 cm²) did not have significant effects on a suite of variables related to midge development from larva to adult, but a higher dose of oil (100 µL on ~785 cm^2) resulted in decreased numbers of larvae pupating and fewer pupae successfully emerging from the water surface as adults. These findings suggest that fog oil deposition may prevent some emergent aquatic insects from completing their life cycle. This is particularly important given that these insects make comprise a large part of the diet of some bats and birds.

5.7 Laboratory Assessment of Photolyzed Fog Oil Toxicity

Photolysis of FO on water dramatically increases the toxicity of FO and increases the amount of water soluble components. At the FO levels observed in the field, photolysis does not increase the toxic effects of the water beneath the oil layer. Only at much higher FO concentrations does the water become highly toxic. Fountain darter larvae are sensitive to relatively low concentrations (e.g., 10 ppm) of photolyzed FO in water. Darter adults, eggs, and juvenile fish are much less sensitive. The length of time of this increased sensitivity is unknown but none the less limited to the time of emergence from the egg until some point in physiological development to juvenile status.

5.8 Analysis of Fog Oil

Military fog oils are very complex hydrocarbon mixtures composed of a multitude of chemically similar components, making complete analysis and characterization difficult, if not impossible. Two-dimensional chromatography, despite its enhanced resolution over one dimensional gas chromatography, is not able to completely characterize such samples. This work, however, shows the value of the extended separation space of GCxGC for group type analysis of FO. Together with a pre-analysis fractionation procedure, many of the chemical classes of FO are revealed. This analysis can be used to verify the efficiency of the FO refinement process and can ensure the elimination of many of the carcinogenic species of concern.

The complementary information derived from using two different column sets is noteworthy and the additional effort is justified depending upon the desired information. The behavior of chemical classes using the conventional column set is well studied and petroleum analysis under these conditions is documented. This column set can easily provide a unique fingerprint for a FO and may prove valuable in difficult applications such as separating refined oil from natural oils and visualizing changes in oil composition after weathering. The inverse column set is better at separating fractions into discrete bands, especially after fractionation. With these two column sets, this work has shown the absence of PAH in military FO, the changes in bulk FO due to the additional processing during manufacturing, and the ability of GCxGC to assign a particular separation pattern to a FO. While only discrete, resolved peaks could be identified with certainty with comparison to a standard, the addition of mass spectral identification would be extremely powerful.

5.9 General Conclusions

The use of oil, generally vegetable oil, to kill predatory aquatic invertebrates in fish hatchery nursery ponds is a common practice. The oil interferes with oxygen transfer between the atmosphere and water and the absorption of oxygen by some aquatic invertebrates. Since FO was demonstrated in Cropek et al. (2008) to be toxic to daphnids, a common food for many larval fishes, and to larval and juvenile stages of a fish, the fountain darters in this study, its use near bodies of water containing aquatic invertebrates and fishes of concern should be limited.

Clearly, the breakdown products that arise due to photolysis is one of the most important factors in determining toxicity of FO to aquatic organisms, and possibly to terrestrial species as well. Since FO is so complex, however, only qualitative comparison of these breakdown products can be performed, but the increase in oxidized, water-soluble compounds was expected and our hypothesis that these compounds would increase the toxic effect of FO was supported. Continued research on FO toxic effects must include the photolytic fraction.

Based upon the results of this study, we conclude that fog oil toxicity to aquatic organisms in the field, while measurable, is low and preventable provided that the generation point is located greater than 50 m from a water body containing threatened or endangered species or their prey items. At this distance, even an hour of fogging does not cause toxicity to sensitive test organisms. Further, although photolysis can increase toxicity, these concentrations of FO are still not great enough to produce a toxic effect, even in our small volume microcosms. The distance pro-

tection can be enhanced by refraining from fog oil use during periods when larvae of endangered fish are most likely to be present. Finally, our work shows that continued buildup of FO on a controlled volume of water can eventually result in a concentration that proves toxic to fish larvae, but this is unlikely in the field where continual water exchange occurs and the environmental water volume is much greater. Conservatively, we suggest that training exercises be limited to five consecutive days.

We are still pursuing a transition document for installations and field managers summarizing the conclusions of this work. Recommendations for methodologies for FO collection in the field are documented in PWTB 200-01-50.

5.10 Future Work Suggestions

This report describes the results of investigations on the effects of military fog oil obscurant, some colored smokes, and mixtures of fog oil and graphite on various trophic and phylogenetic levels of aquatic plants and animals, particularly as may be relevant to threatened or endangered vertebrates. The results generally indicate a minimal level of toxicity under the conditions tested. With appropriate management of fog oil obscurant and S&O releases in typical field training exercises, as will be addressed in developing usage guidance documents, these compounds appear to cause minimal risk to aquatic environments.

However, this effort dealt with representative but not all life forms. Future work should focus those species groups for which little or nothing is currently known. For example, while fountain darters, in part because of known environmental sensitivities and endangered species status, are excellent fish for study, other groups such as members of the common Cyprinidae (e.g., Topeka shiner) and economically and socially important and environmentally sensitive Salmonidae (e.g., rainbow trout), should be similarly studied. These species would be representative of those inhabiting diverse and differing environments, with perhaps different sensitivities. Also, while some preliminary investigation of the effects of S&O on leopard frogs was done as a part of this work, given broader scientific and environmental concern regarding the national and international status of amphibians, further investigation is called for. This work also provided preliminary information of the effects of S&O of submersed plant forms. Emergent aquatic and wetland plant species would be exposed to fog oil obscurant and other S&O in a different fashion, and should be similarly investigated. These exposures, along with different plant characteristics and ecological importance, may produce different results.

Because a number of the compounds found in S&O may bioaccumulate in tissues, it would be instructive to investigate the food-borne exposure of fish to S&O related contaminants. These experiments will allow us to directly measure the indirect effects of S&O on TE fish upon consumption of exposed midges rather than relying on results of midge exposure experiments to estimate indirect effects on fish. Two different experiments are proposed. The first will involve exposing early instar larval midges (*Chironomus tentans*) to various S&O at different sub-lethal concentrations as determined in previous experiments. After exposure, midges will be presented to unexposed fish (fathead minnows, *Pimephales promelas*). Numbers of exposed midges consumed will be documented and compared to numbers of unexposed midges consumed by fish. After a predetermined period of time, each set of fish will be sacrificed, evaluated for gona-

dosomatic index (GSI), and tissue contaminant residue levels will be determined. A second set of experiments will involve exposing fish to various S&O at different sub-lethal concentrations and flow rates and then offering them unexposed midges. Experimental endpoints will include prey capture efficiency and fish tissue residue levels. Residue levels found in directly exposed fish will be compared to those in controls as well as to those in unexposed fish fed exposed midges.

We also suggest that closer examination of ultimate S&O sinks in the environment should be performed. In our experiments, there is little toxic effect of FO at the short durations (days) employed here but years of environmental exposure to S&O may have an accumulative effect.

Our data have also shown that during the release of colored smoke dye, the high temperature pyrolytic effects can alter the dye into by-products that may be more toxic than the starting dye. New formulations of grenade ingredients should study the pyrolytic breakdown products during performance evaluation to ensure that these grenades are not introducing problematic chemicals into the environment.

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APPENDIX A: SUPPORTING DATA

The three tables comprising this appendix summarize the exposure results for species of interest to the various smokes and obscurants as specified in the table captions. Note: in all three tables, "NS" means that there was no significant difference in acute mortality from the control specimens 5 m upwind.

Table A1. Results of exposure of *R. pipiens*, *P. promelas*, *E. fonticola*, *N. Topeka*, *O. mykiss*, and *C. tentans* to colored smokes at field concentrations (control 5 meters upwind).

Smoke	No. Grenades	Species	Exposure distance (meters)							
			1,	5,	25,	50,	100,	250,	500,	800
Red (May)	1	*P. promelas* (ad)	-	NS	NS	NS	NS	NS	NS	NS
		P. promelas (fry)	-	NS	NS	NS	NS	NS	NS	NS
		R.. pipiens (larvae)	-	NS	NS	NS	NS	NS	NS	NS
		C. tentans (larvae)	-	NS	NS	NS	NS	NS	NS	NS
Red (May)	6	*P. promelas* (ad)	-	NS	NS	NS	NS	NS	NS	NS
		P. promelas (fry)	-	NS	NS	NS	NS	NS	NS	NS
		R.. pipiens (larvae)	-	NS	NS	NS	NS	NS	NS	NS
		C. tentans (larvae)	-	NS	NS	NS	NS	NS	NS	NS
Red (Dec)	19	*N. Topeka* (ad)	NS	NS	-	-	-	-	-	-
		O. mykiss (juv)	NS	NS	-	-	-	-	-	-
Red (Aug)	20	*E. fonticola* (ad)	-	NS	NS	-	-	-	-	-
		E. fonticola (fry)	-	NS	NS	-	-	-	-	-
		R. pipiens (larvae)	-	NS	NS	-	-	-	-	-
Green (May)	1	*P. promelas* (ad)	-	NS	NS	NS	NS	NS	NS	NS
		P. promelas (fry)	-	NS	NS	NS	NS	NS	NS	NS
		R.. pipiens (larvae)	-	NS	NS	NS	NS	NS	NS	NS
		C. tentans (larvae)	-	NS	NS	NS	NS	NS	NS	NS
Green (May)	7	*P. promelas* (ad)	NS	NS	NS	NS	NS	NS	NS	NS
		P. promelas (fry)	NS	NS	NS	NS	NS	NS	NS	NS
		R.. pipiens (larvae)	NS	NS	NS	NS	NS	NS	NS	NS
		C. tentans (larvae)	NS	NS	NS	NS	NS	NS	NS	NS
Green (Aug)	20	*E. fonticola* (ad)	-	NS	NS	-	-	-	-	-
		E. fonticola (fry)	-	NS	NS	-	-	-	-	-
		R. pipiens (larvae)	-	NS	NS	-	-	-	-	-
Green (Dec)	22	*N. Topeka* (ad)	-	NS	NS	-	-	-	-	-
		O. mykiss (juv)	-	NS	NS	-	-	-	-	-
Yellow (May)	1	*P. promelas* (ad)	-	NS	NS	NS	NS	NS	NS	NS
		P. promelas (fry)	-	NS	NS	NS	NS	NS	NS	NS
		R.. pipiens (larvae)	-	NS	NS	NS	NS	NS	NS	NS
		C. tentans (larvae)	-	NS	NS	NS	NS	NS	NS	NS
Yellow (May)	7	*P. promelas* (ad)	-	NS	NS	NS	NS	NS	NS	NS
		P. promelas (fry)	-	NS	NS	NS	NS	NS	NS	NS

Smoke	No. Grenades	Species	Exposure distance (meters) 1,	5,	25,	50,	100,	250,	500,	800
		R.. pipiens (larvae)	-	NS	NS	NS	NS	NS	NS	NS
		C. tentans (larvae)	-	NS	NS	NS	NS	NS	NS	NS
Yellow (May)	16	*P. promelas* (ad)	NS	-	-	-	-	-	-	-
		P. promelas (fry)	NS	-	-	-	-	-	-	-
		R.. pipiens (larvae)	NS	-	-	-	-	-	-	-
		C. tentans (larvae)	NS	-	-	-	-	-	-	-
Yellow (Aug)	20	*E. fonticola* (ad)	-	NS	NS	-	-	-	-	-
		E. fonticola (fry)	-	NS	NS	-	-	-	-	-
		R. pipiens (larvae)	-	NS	NS	-	-	-	-	-
Yellow (Dec)	20	*N. Topeka* (ad)	NS	NS	-	-	-	-	-	-
		O. mykiss (juv)	NS	NS	-	-	-	-	-	-

Note: “NS” indicates that there was no significant difference in acute mortality from controls.

Table A2. Results of exposure of *R. pipiens*, *P. promelas*, *E. fonticola, N. Topeka*, *O. mykiss*, and *C. tentans* to fog oil obscurant at field concentrations (control 5 meters upwind).

Obscurant	Duration (min.)	Species	Exposure distance (meters) 1,	5,	25,	50,	100,	250,	500,	800
Fog Oil (May)	3	*P. promelas* (ad)	-	NS	NS	NS	NS	NS	NS	NS
		P. promelas (fry)	-	NS	NS	NS	NS	NS	NS	NS
		R.. pipiens (larvae)	-	NS	NS	NS	NS	NS	NS	NS
		C. tentans (larvae)	-	NS	NS	NS	NS	NS	NS	NS
Fog Oil (Aug)	3	*E. fonticola* (ad)	-	NS	NS	NS	NS	NS	-	-
		E. fonticola (fry)	-	NS	NS	NS	NS	NS	-	-
		R.. pipiens (larvae)	-	NS	NS	NS	NS	NS	-	-
Fog Oil (May)	18	*P. promelas* (ad)	-	NS	NS	NS	NS	NS	NS	NS
		P. promelas (fry)	-	NS	NS	NS	NS	NS	NS	NS
		R.. pipiens (larvae)	-	NS	NS	NS	NS	NS	NS	NS
		C. tentans (larvae)	-	NS	NS	NS	NS	NS	NS	NS
Fog Oil (Aug)	18	*E. fonticola* (ad)	-	NS	NS	NS	NS	NS	NS	-
		E. fonticola (fry)	-	NS	NS	NS	NS	NS	NS	-
		R.. pipiens (larvae)	-	NS	NS	NS	NS	NS	NS	-
Fog Oil (Dec)	18	*N. Topeka* (ad)	-	NS	NS	NS	NS	-	-	-
		O. mykiss (juv)	-	NS	NS	NS	NS	-	-	-
Fog Oil (Aug)	30	*E. fonticola* (ad)	-	NS	NS	NS	NS	NS	NS	NS
		E. fonticola (fry)	-	NS	NS	NS	NS	NS	NS	NS
		R.. pipiens (larvae)	-	NS	NS	NS	NS	NS	NS	NS
Fog Oil (Dec)	30	*N. Topeka* (ad)	-	NS	NS	NS	NS	-	-	-
		O. mykiss (juv)	-	NS	NS	NS	NS	-	-	-
Fog Oil (Aug)	60	*E. fonticola* (ad)	-	NS	NS	NS	NS	NS	NS	NS
		E. fonticola (fry)	-	NS	NS	NS	NS	NS	NS	NS
		R.. pipiens (larvae)	-	NS	NS	NS	NS	NS	NS	NS
Fog Oil (Dec)	60	*N. Topeka* (ad)	-	NS	NS	NS	NS	-	-	-
		O. mykiss (juv)	-	NS	NS	NS	NS	-	-	-
Fog Oil (Aug)	120*	*E. fonticola* (ad)	-	NS	NS	NS	NS	NS	NS	NS
		E. fonticola (fry)	-	NS	NS	NS	NS	NS	NS	NS
		R.. pipiens (larvae)	-	NS	NS	NS	NS	NS	NS	NS
Fog Oil (Dec)	120*	*N. Topeka* (ad)	-	NS	NS	NS	NS	-	-	-
		O. mykiss (juv)	-	NS	NS	NS	NS	-	-	-

* nominal time

Note: "NS" indicates that there was no significant difference in acute mortality from controls.

Table A3. Results of exposure of *R. pipiens*, *P. promelas*, *E. fonticola, N. Topeka*, *O. mykiss*, and *C. tentans* to fog oil obscurant and graphite combination at field concentrations (control 5 meters upwind).

Obscurant	Duration (min.)	Species	Exposure distance (meters) 1,	5,	25,	50,	100,	250,	500,	800
Fog Oil and Graphite (Aug)	3	*E. fonticola* (ad)	-	NS	NS	NS	NS	NS	-	-
		E. fonticola (fry)	-	NS	NS	NS	NS	NS	-	-
		R.. pipiens (larvae)	-	NS	NS	NS	NS	NS	-	-
Fog Oil and Graphite (May)	13:35	*P. promelas* (ad)	-	-	-	NS	-	-	-	-
		P. promelas (fry)	-	-	-	NS	-	-	-	-
		R.. pipiens (larvae)	-	-	-	NS	-	-	-	-
		C. tentans (larvae)	-	-	-	NS	-	-	-	-
Fog Oil and Graphite (Aug)	18	*E. fonticola* (ad)	-	NS	NS	NS	NS	NS	NS	-
		E. fonticola (fry)	-	NS	NS	NS	NS	NS	NS	-
		R.. pipiens (larvae)	-	NS	NS	NS	NS	NS	NS	-
Fog Oil and Graphite (Dec)	18	*N. Topeka* (ad)	-	NS	NS	NS	NS	-	-	-
		O. mykiss (juv)	-	NS	NS	NS	NS	-	-	-
Fog Oil and Graphite (Dec)	30	*N. Topeka* (ad)	-	NS	NS	NS	NS	-	-	-
		O. mykiss (juv)	-	NS	NS	NS	NS	-	-	-
Fog Oil and Graphite (Aug)	60	*E. fonticola* (ad)	-	NS	NS	NS	-	NS	NS	-
		E. fonticola (fry)	-	NS	NS	NS	-	NS	NS	-
		R.. pipiens (larvae)	-	NS	NS	NS	-	NS	NS	-
Fog Oil and Graphite (Dec)	60	*N. Topeka* (ad)	-	NS	NS	NS	NS	-	-	-
		O. mykiss (juv)	-	NS	NS	NS	NS	-	-	-
Fog Oil and Graphite (Aug)	120*	*E. fonticola* (ad)	-	NS	NS	NS	NS	NS	NS	-
		E. fonticola (fry)	-	NS	NS	NS	NS	NS	NS	-
		R.. pipiens (larvae)	-	NS	NS	NS	NS	NS	NS	-
Fog Oil and Graphite (Dec)	120*	*N. Topeka* (ad)	-	NS	NS	NS	NS	-	-	-
		O. mykiss (juv)	-	NS	NS	NS	NS	-	-	-

* nominal time

Note: "NS" indicates that there was no significant difference in acute mortality from controls.

APPENDIX B: LIST OF TECHNICAL PUBLICATIONS PRODUCED FOR THIS PROJECT

Journal Articles:

Cropek D. M., Esarey J. C., Conner C. L., Goran J. M., Smith T. S., and Soucek D. J. 2008. Toxicological effects of fog oil obscurant on *Daphnia magna* and *Ceriodaphnia dubia* in field and laboratory exposures. *Ecotoxicology*. ISSN 0963-9292 (Print), 1573-3017 (Online), http://www.springerlink.com/content/f5737w3344542u59/ (accessed 29 April 2008).

Smith T. S., Cropek D. M., Lembi C., Soucek D. J., and Wilkinson K. Response of Stuckenia pectinatus and Selanasturm capricornutum to field exposure to military fog oil obscurant. *Wetlands Ecology and Management.* ISSN 0923-4861 (Print), 1572-9834 (Online), in preparation. http://www.springer.com/life+sci/ecology/journal/11273 (accessed 29 April 2008).

T. A. Ryan , A. N. Kohl, D. J. Soucek, T. S. Smith, T. M. Brandt, D. M. Cropek Short Term Effects of Military Fog Oil on the Fountain Darter *(Etheostoma fonticola)*, Bulletin of Environmental Contamination and Toxicology, in preparation.

Donald M Cropek and Anja Kohl. Comprehensive Two-dimensional Chromatography of Military Fog Oils, J Chromatogr. A, in preparation.

Conference Proceedings and Non-Refereed Publications:

Headquarters, U.S. Army Corps of Engineers (HQUSACE). 2007. Comparison of solid substrates for collecting military smoke and obscurant deposition, Public Works Technical Bulletin (PWTB) 200-01-50, 1 September 2007 http://www.wbdg.org/ccb/ARMYCOE/PWTB/pwtb_200_1_50.pdf (accessed 29 April 2008).

Cropek D. M., Smith T. S., Williams R., and Soucek D. J. Military smokes and obscurants formulations. White Paper submitted to Dept of Defense's Strategic Environmental Research and Development Program. 3 June 2003, 18 pp.

Esarey J. C., Soucek D. J, Cropek D. M., and Smith T. Toxicological effects of military smokes and obscurants on aquatic threatened and endangered species. Proceedings of the 24th Army Science Conference, Orlando, FL. 29 Nov - 2 Dec 2004.

Cropek D.M. Two-dimensional Gas Chromatography / FID Analysis of Military Fog Oils. Presentation at EnviroAnalysis 2007 Conference, Wellington, New Zealand, 7-9 February 2007 http://www.cmsl.co.nz/default,518.sm accessed 29 April 2008).

Invited Seminars:

Cropek D. M., Soucek D. J., Esarey J. C., Smith T. S., Rush T., Williams R., Conway B., Lembi C., and Furnari D. Toxicological Effects of Military Smokes and Obscurants on Aquatic Threatened and Endangered Species. Invited Seminar at the Strategic Environmental Defense Program and Environmental Security and Technology Certification Program combined Symposium, Washington, DC. 29 Nov - 2 Dec 2004.

Smith T. S., Soucek D. J., Cropek D. M. Toxicological effects of military smokes and obscurants on selected species. U.S. Fish and Wildlife Service training workshop on Monitoring and Adaptive Management for Endangered Species Conservation, Shepherdstown, WV. September 13- 17, 2004.

Cropek D.M. and A. Kohl, Comprehensive Two-Dimensional Gas Chromatography of Military Fog Oils, Pittsburgh Conference, New Orleans, March 2008.

Kohl A. and Cropek D.M. Toxicological Effects of Smoke and Obscurants on Aquatic Threatened and Endangered Species, 16th General Meeting of the US/GE Environmental Technology DEA, Erding, Germany, 9 Apr 2008.

Contributed Presentations/Published Abstracts:

Smith, T. S., Cropek D. M., Soucek D. J., Curtin D., and Williams R. 2007. Military Smoke and Obscurant Use In Training - Support for Continued Use and in NEPA and ESA Analysis. Presented at 2007 US Army Sustainable Range Program Conference, Hampton, VA, 15 May 2007.

Cropek, D. M., T. Smith, C.L. Conner, T. Ryan, D. J. Soucek, and J. C. Esarey, Toxicity of the Military Obscurant, Fog Oil, to Aquatic Species, Poster presentation at the Strategic Environmental Defense Program and Environmental Security and Technology Certification Program combined Symposium, Washington, DC, December 1 2006.

Cropek DM, Soucek DJ, Esarey JC, Smith T, Conner C. Toxicity of the military obscurant, fog oil, to aquatic prey species. Poster presentation at the Strategic Environmental Defense Program and Environmental Security and Technology Certification Program combined Symposium, Washington, DC. December 2005.

Esarey JC, Soucek DJ, Cropek DM, Smith T. Toxicity of the military obscurant, fog oil, to Daphnia magna. Poster presentation at the 26th annual meeting of the Society of Environmental Toxicology and Chemistry, Baltimore, MD, November 13-17, 2005.

Esarey JC, Soucek DJ, Cropek DM, Smith T. Toxicological effects of military fog oil obscurant to Daphnia magna in field exposures. Platform presentation at the Technical Symposium & Workshop on Threatened, Endangered, and At-Risk Species on DoD and Adjacent Lands, Baltimore, MD. June 7-9, 2005.

Cropek DM, Soucek DJ, Esarey JC, Smith T, Rush T, Williams R, Conway B, Lembi C, Furnari D. Toxicological effects of military smokes and obscurants on aquatic threatened and endangered species. Poster presentation at the Strategic Environmental Defense Program and Environmental Security and Technology Certification Program combined Symposium, Washington, DC. 29 Nov - 2 Dec 2004.

Cropek DM, Soucek DJ, Esarey JC, Smith T, Rush T, Williams R, Conway B, Lembi C, Furnari D. Toxicological effects of military smokes and obscurants on aquatic threatened and endangered species, Poster presentation at the 24th Army Science Conference, Orlando, FL. 29 Nov - 2 Dec 2004.

Smith T, Cropek DM, Soucek DJ, Brandt T, Kerns H, Nolde C. Toxicological effects of military smokes and obscurants on selected fish species. Platform presentation at the Annual Meeting of the American Fisheries Society, Madison, WI. August 22-26, 2004.

Smith T, Cropek DM, Soucek DJ. Fog oil and other smoke and obscurant effects on threatened and endangered species. Platform presentation at the U.S. Army National Guard Sustainable Range Conference, Camp Blanding, FL. 29 Mar - 2 Apr 2004.

Cropek D. M., Soucek D. J., Smith T. S., Williams R., Lembi C. and Furnari D. Toxicological effects of smokes and obscurants on aquatic threatened and endangered species. Poster presented at the Partners in Environmental Technology Technical Symposium & Workshop, Washington, DC, December 2-4, 2003.